"TO CO-RELATE SERUM IRON AND SERUM FERRITIN IN RELATION TO CHOLELITHIASIS- A ONE YEAR CROSS SECTIONAL OBSERVATIONAL STUDY"

By

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ABSTRACT

Background:

- Cholelithiasis is a prevalent medical condition that affects both men and women. It poses a considerable burden on healthcare systems globally and is one of the most frequent issues among patients who visit emergency rooms due to abdominal pain.
- The traditional belief that overweight, fertile women in their forties are most susceptible to gallstones is only partially accurate, as the disease has also been observed in thin and underweight individuals, as well as in women post-delivery.
- Recent research has shown that there is a positive correlation between cholelithiasis and trace elements (such as calcium, iron, zinc, and copper) as well as abnormal pH levels.

AIMS AND OBJECTIVES:

- To co-relate the association of serum iron and serum ferritin in relation to cholelithiais
- To co-relate the association of anaemia with cholelithiasis

METHODS:

- This was a prospective cross sectional observational study conducted in Department of General Surgery in KLES Dr Prabhakar Kore Hospital and Medical Research Centre, KAHER, Belagavi for duration of one year. Demographic data, clinical characteristic, investigations of total 85 patients who were more than 18 years of age diagnosed with cholelithiasis.
- Serum iron and Serum ferritin along with Haemoglobin was collected at the time of admission after diagnosing with cholelithiasis on USG or CT abdomen.

- Patients were characterized into Low, Normal and High values according to the gender of the study population.

RESULTS

- Males and females were both included in the study where females outnumbered males and patients ranged from 18-90 years.
- 41.18% of study group of population were found to have low serum iron levels and majority of them were females. 57.65% of study group of population had normal serum iron levels.
- 11.76% of study group of population had low serum ferritin levels and majority of them were female group of population. 71.76% of study group of population had normal serum ferritin levels.
- 57.65% of study group of population had low haemoglobin levels and majority of them were female group of population. 41.18% of total study population had normal haemoglobin levels.
- On comparing the serum iron, serum ferritin and haemoglobin values, low serum iron, low serum ferritin and low haemoglobins was statistically more significant in female group of population than males.

CONCLUSION

- Iron deficiency anaemia is a significant finding in the female group of population leading to supersaturation of bile and subsequent cholelithiasis.
- There is a definite co-relation between serum iron, serum ferritin and haemoglobin levels in female group of patients diagnosed with cholelithiasis.
- Hence, screening for these three parameters in further studies can help towards prevention and better management of patients, with calculous cholelithiasis.

LIST OF ABBREVIATIONS

GLOSSARY	ABBREVIATIONS
GB	Gall Bladder
CHD	Common Hepatic Duct
CD	Cystic Duct
CBD	Common Bile Duct
CA	Cystic Artery
RBC	Red Blood Cells
UDP	Uridine Diphosphate
NOS	Nitric Oxide Synthase
NO	Nitric Oxide
nNOS	Neuronal Nitric Oxide Synthase
eNOS	Endothelial Nitric Oxide Synthase
iNOS	Inducible Nitric Oxide Synthase
NANC	Non Adrenergic Non cholinergic Neurotransmitter
L-NAME	Nv-Nitro-L-Methyl Ester
CO2	Carcon-di-oxide
CT	Computed Tomography
USG	Ultrasonography
MRC	Medical Research Centre
KAHER	KLE Academy of Higher Education and Research
KLE	Karnataka Lingayat Education

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INTRODUCTION

- Gallstone disease, or cholelithiasis, is indeed a prevalent condition globally, imposing a significant burden on healthcare systems and affecting individuals across different demographics. It ranks among the most common reasons for emergency room visits due to abdominal discomfort. In India, its prevalence ranges from 6% to 9% among adults.
- While the conventional perception was that overweight, fertile women in their forties were most susceptible to gallstones, recent research has challenged this notion. Thin or underweight individuals, as well as women post-delivery, have also been found to develop gallstones. Studies have indicated a positive correlation between cholelithiasis and trace elements such as calcium, iron, zinc, and copper, as well as abnormalities in pH levels.
- However, the role of iron in causing cholesterol supersaturation in bile, a key factor in gallstone formation, remains uncertain. Hence, there is a pressing need for further research to elucidate the contributions of these elements to cholelithiasis. Understanding these mechanisms can inform the development of effective preventive measures aimed at reducing the incidence of gallstone disease and its associated complications.

OBJECTIVE OF STUDY

PRIMARY OBJECTIVE

To co-relate the association of serum iron and serum ferritin in relation cholelithiais

SECONDARY OBJECTIVE

To co-relate the association of anaemia with cholelithiasis

REVIEW OF LITERATURE

A.EMBRYOLOGY[11]

In the early stages of embryonic development, a protrusion emerges from the ventral wall of the primitive midgut around the fourth week of pregnancy. Here's a breakdown of the key developmental milestones:

1. At the 3-milimeters stage: A cranial bud forms, which later matures into the liver lobes. Concurrently, the caudal bud gives rise to the gallbladder and extrahepatic biliary system. By day 26, a cystic diverticulum emerges from the caudal duct, eventually developing into gall bladder and cystic duct by the end of the fourth week.
2. Progressing to the 5-milimeters stage: The common hepatic duct takes shape from a portion of the hepatic pars located distally to the cystic pars, while the common bile duct originates from the segment between the cystic pars and duodenal segment of the foregut.
3. Advancing to the 7-milimeters stage: The liver, hepatic ducts, gallbladder, cystic duct, and ventral pancreas begin to take form.
4. By the 12-milimeters stage: The ventral pancreatic bud undergoes a 180-degree clockwise rotation around the duodenum, resulting in the assimilation of the ventral and dorsal buds. This fusion completes formation of the pancreas, typically occurring by the 6th or 7th week of gestation.

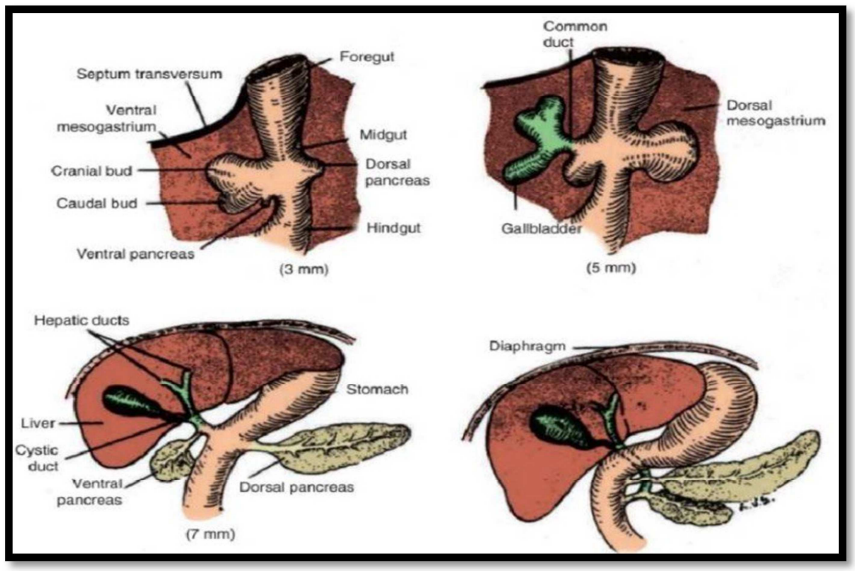


FIGURE 1: EMBRYOLOGICAL DEVELOPMENT OF EXTRAHEPATIC BILIARY TREE

A. SURGICAL ANATOMY

1. GALLBLADDER(GB)

The gallbladder is a sac-shaped organ, typically measuring 7-10 centimeters in length, situated in an anatomical depression on the underside of the liver. Its average capacity ranges from 30 to 50 milliliters, although it can expand to hold up to 300 milliliters in cases of blockage of bile outflow.[8]

Anatomically, the gallbladder can be divided into four main parts:

a) Fundus: This portion is round in shape and extends beyond 2 centimeters below the lower margin of the liver, terminating in a blind end.

b) Body: Comprising elastic tissue, the body of the gallbladder is responsible for its distensibility, allowing it to expand as needed.

c) Infundibulum: Located at the junction of cystic duct and neck, the infundibulum forms a mucosal outpouching.

d) Neck: Positioned at the deepest part of the gallbladder fossa, the neck serves as the connection point to the cystic duct.

Peritoneal lining of liver extends to cover fundus and inferior surface of gall bladder[9]

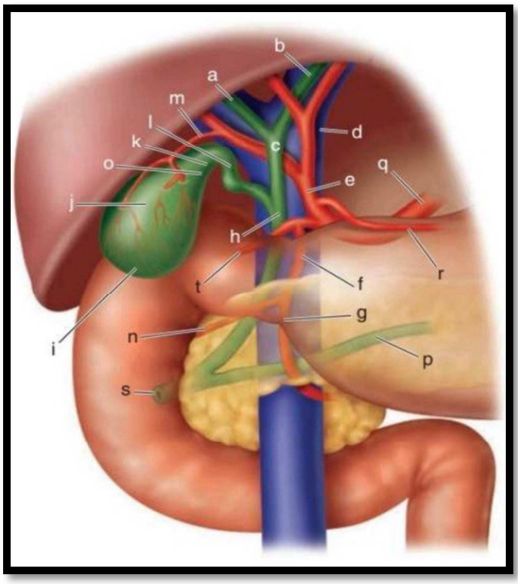


FIGURE 2: BILIARY ANATOMY :

A. Right hepatic Artery
B. left Hepatic Duct
C. Common hepatic duct
D. Portal vein
E. Hepatic artery Proper
F. Gastroduodenal artery
G. Right gastroepiploic artery
H. Common bile duct
I. Fundus of gallbladder
J. Body of gallbladder
K. Infundibulum of gallbladder
L. Cystic duct
M. Cystic artery
N. Superior pancreaticoduodenal artery
O. Neck of GB
P. Pancreatic duct
Q. Common hepatic artery
R. Right gastric artery
S. Ampulla of vater
T. Supraduodenal artery

2. EXTRAHEPATIC BILIARY TREE

a) Hepatic duct: The right hepatic duct is shorter than left hepatic duct.The left hepatic duct is more likely to get dilated in cases of obstruction at its distal end

b) Common hepatic duct: It is formed by merging of the right and left hepatic ducts near the surface of the liver, the CHD measures between 1 to 4 centimeters in length and has a diameter of approximately 4 millimeters. It is positioned to the right of hepatic artery and anterior to portal vein.

c) Cystic duct: It emerges from the gallbladder, CD varies in length and course. It features spiral folds in its mucosa, known as the valves of Heister.

d) d. Common bile duct: Confluence of common hepatic duct and cystic duct at an acute angle results in formation of CBD. It measures between 7 to 11 centimeters in length and has a diameter ranging from 5 to 10 millimeters

The parts of the bile duct system can be classified as follows:

1. Supraduodenal: This segment spans approximately 2.5 centimeters and is situated to right of hepatic artery, running along the free edge of lesser omentum and anterior to portal vein.
2. Retroduodenal: Positioned posterior to first part of the duodenum, this segment moves away from portal vein and hepatic artery.
3. Infraduodenal: Found on the posterior surface of pancreatic head, this segment passes through the pancreatic tissue.
4. Intraduodenal: This segment merges with the pancreatic duct and terminates by opening into ampulla of Vater within the second part of the duodenum.[9]

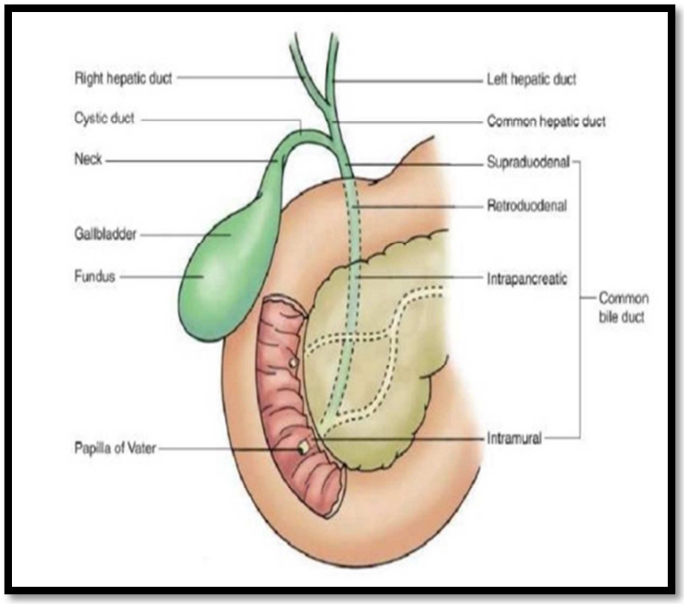


FIGURE 3 : ANATOMICAL DIVISION OF BILIARY SYSTEM

SPHINCTER OF ODDI

The sphincter of Oddi, ranging from 4 to 6 millimeters in length, is regulated by interstitial cells of Cajal[8,9]. Composed of thick circular muscle fibers, it encircles common bile duct at ampulla of Vater. Its functions include:

1. Prevention of the backflow of duodenal contents into biliary tree.
2. Regulation of flow of pancreatic and biliary juices.
3. Diversion of bile to gallbladder during fasting periods.

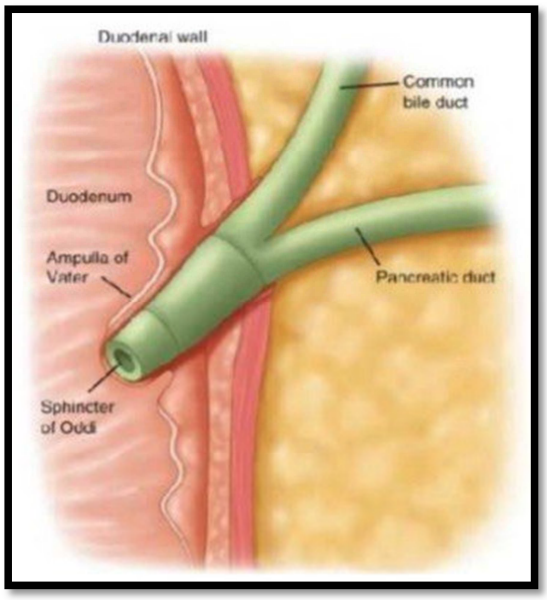


FIGURE 4 : SPHINCTER OF ODDI

3. HISTOLOGY OF GALLBLADDER

a. Mucosa: Comprised of single layer of simple columnar epithelium containing fat globules and cholesterol. Tubuloalveolar glands within the mucosal lining of the neck and infundibulum secrete mucus.
b. Lamina propria:
c. Muscular layer: Characterized by indistinct circular, longitudinal, and oblique muscle fibers.

d. Adventitia: Composed of connective tissue, vessels, lymphatics, and nerves.

e. Serosa[10]

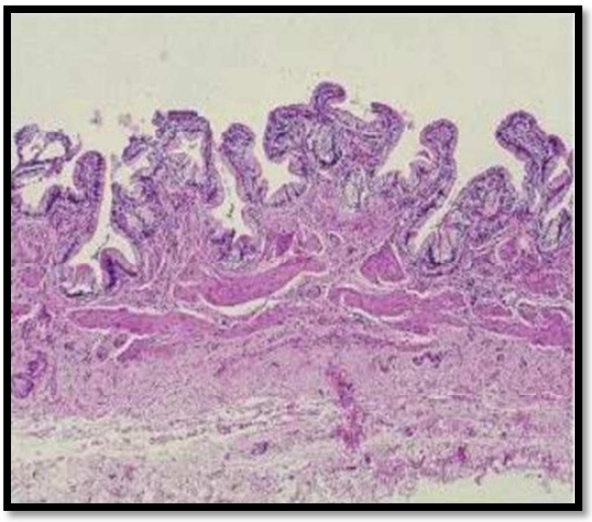


FIGURE 5 : GALL BLADDER HISTOLOGY

4. BLOOD SUPPLY

A.ARTERIAL SUPPLY

The cystic artery exhibits a variable origin, it commonly branching from right hepatic artery. It primarily supplies a significant portion of the gallbladder and is situated in close proximity to CD within Calot's triangle. Its superficial and deep branches, provided at the neck of gallbladder, form anastomoses over the body and fundus of gallbladder.

As the cystic artery serves as an end artery, its occlusion can result in gangrene of the gallbladder[9]

Regarding anomalies, the "caterpillar turn" or "Moynihan’s hump" presents a risky anomaly characterized by a short cystic artery and a tortuous right hepatic

artery. This anomaly poses a potential risk of inadvertent arterial injury or bleeding during cholecystectomy.[9]

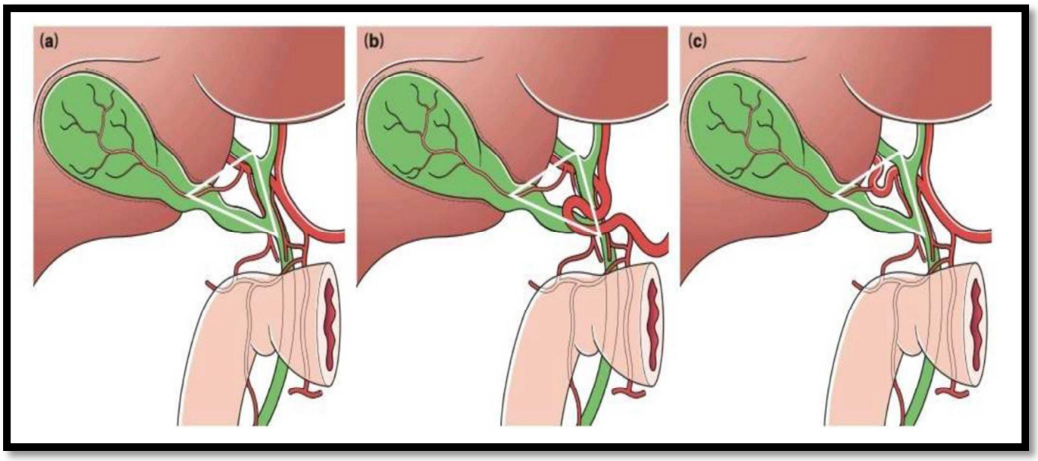


FIGURE 6 : A. CALOT'S TRIANGLE B. TORTUOUS RIGHT HEPATIC ARTERY WITH SHORT CYSTIC ARTERY ; B AND C ARE EXAMPLES OF MOYNIHAN'S HUMP.

B.VENOUS SUPPLY

Veins draining the bile ducts typically empty into the 9 o'clock and 3 o'clock veins along common biliary channel. However, the venous drainage of gallbladder differs. Multiple smaller veins directly transmit blood into tributaries of right portal vein within the liver, running straight from gallbladder through its bed.[10]

5. LYMPHATIC SUPPLY

The cystic lymph node of Lund, situated at junction angle of the cystic and common Hepatic duct, serves as a drainage point for the gallbladder. From there, the efferent vessels of this lymph node carry lymphatic fluid into the liver at its hilum, and further onward into the celiac lymph nodes.

Additionally, the subcapsular lymphatic vessels of the liver merge with the subserosal lymph vessels of the gallbladder, contributing to lymphatic drainage network of hepatic and gallbladder regions**.**[10]

6. ANATOMICAL VARIATIONS

Indeed, awareness of anatomical anomalies in the formation of teh extrahepatic biliary system and gallbladder is crucial for surgeons performing cholecystectomy. [9]

These anomalies can vary widely among individuals and may include variations in the anatomy of structures such as the cystic artery, cystic duct, and bile ducts.

Understanding these variations helps surgeons anticipate potential challenges during surgery and minimize the risk of complications such as inadvertent injury to vital structures, bleeding, or bile leakage. Therefore, thorough preoperative assessment and awareness of possible anatomical anomalies are essential for ensuring the safety and success of cholecystectomy procedures**.**

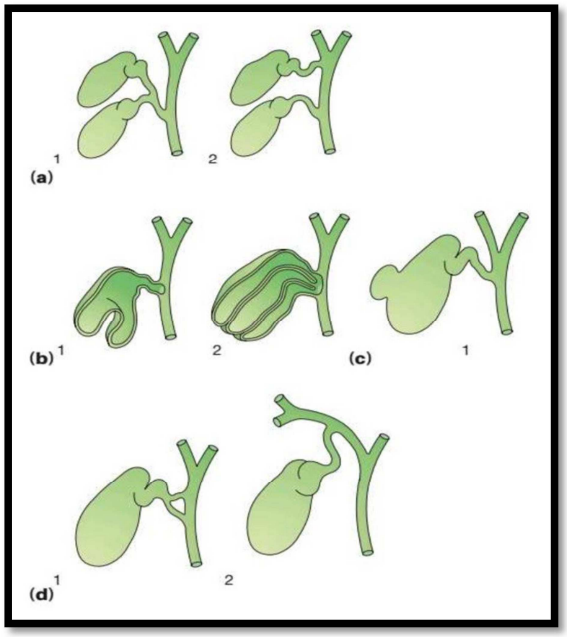


FIGURE 7 : MAIN VARIATIONS IN GALLBLADDER AND CYSTIC DUCT ANATOMY :

a. Double gall bladder
b. Septum of gallbladder
   1. Phrygian cap
c. Diverticulum of gallbladder
d. Variation in cystic duct insertion

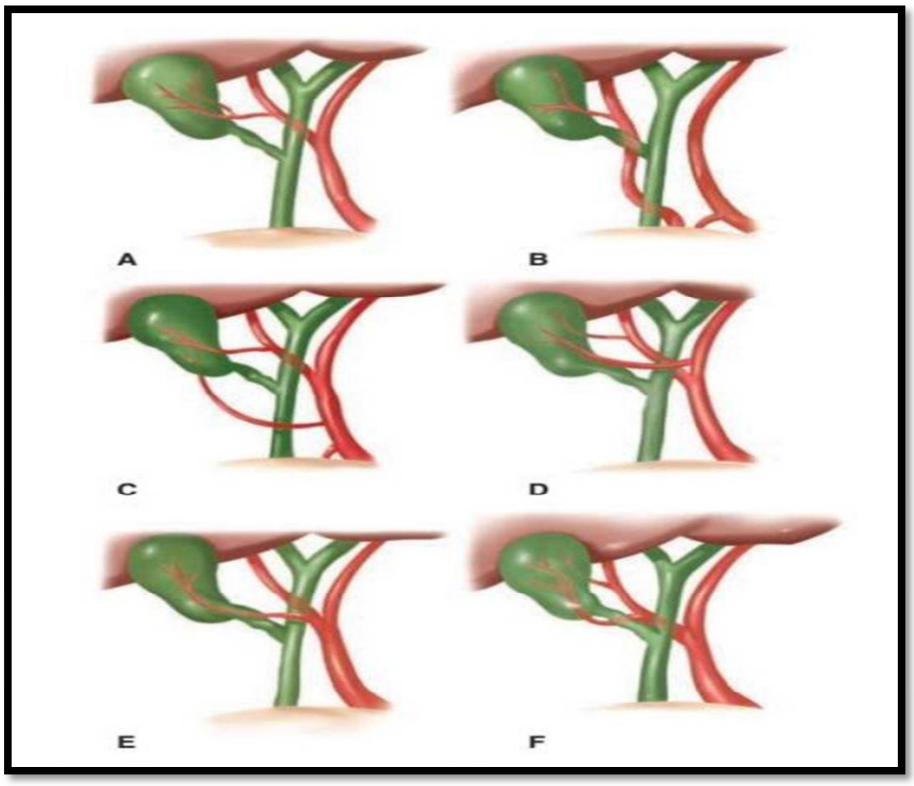


FIGURE 8: VARIATIONS IN CYSTIC ARTERY

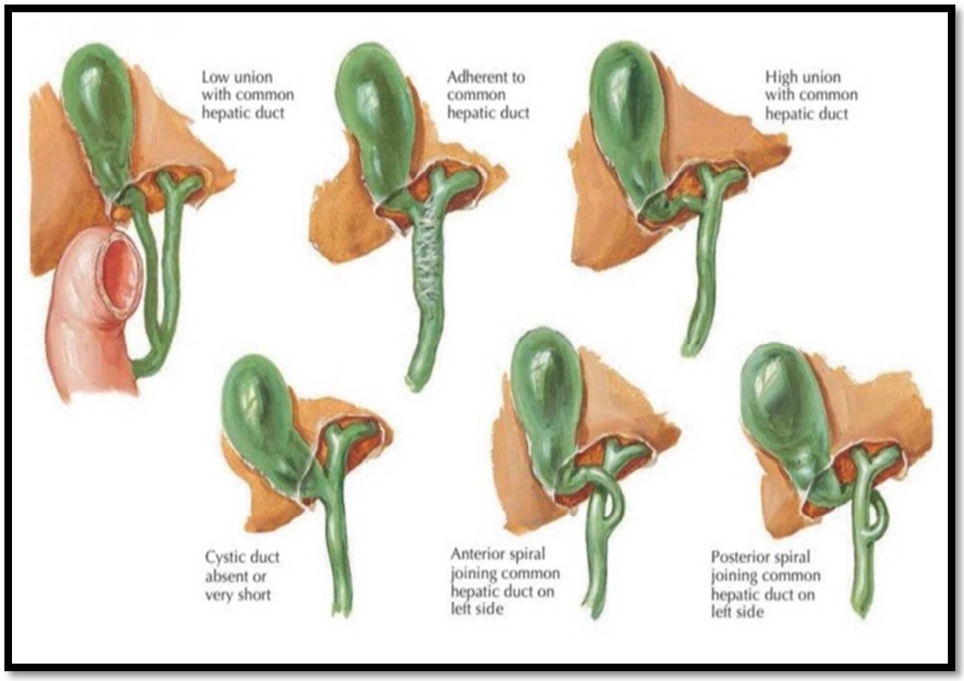


FIGURE 9: VARIATIONS IN CYSTIC DUCT

7. NERVE SUPPLY

Here's a breakdown of the nerve supply to the gallbladder and its associated functions:

1. Parasympathetic nerve supply originates from the anterior vagal trunk.
2. Sympathetic nerve supply, with cell bodies located in the T7-T9 spinal segments, stems from the celiac ganglion.
3. Right infrascapular referred pain is attributed to the right sympathetic fibers, which predominantly transmit afferent pain signals over the T7-T9 spinal segment. Additionally, some afferent fibers from right phrenic nerve traverse the hepatic, phrenic, and coeliac plexuses near the gallbladder, causing referred pain in the right hypochondrium that may radiate to the back between the shoulder blades, particularly in cases of gallstones.
4. The parasympathetic system governs the contraction of the GB and relaxation of ampullary sphincter, while the sympathetic system inhibits contractions of the gallbladder.[9,10]

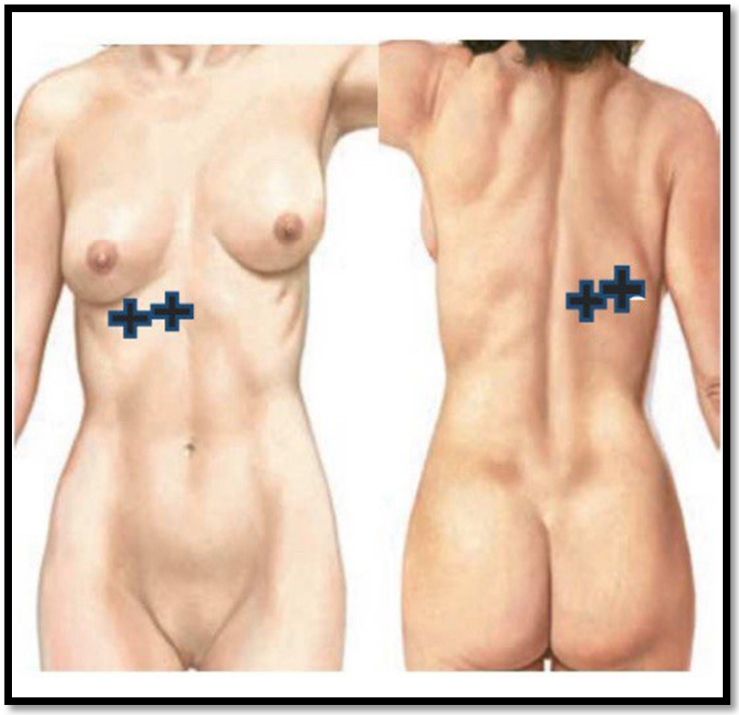


FIGURE 10 : DISTRIBUTION OF PAIN

8.CALOT'S TRIANGLE

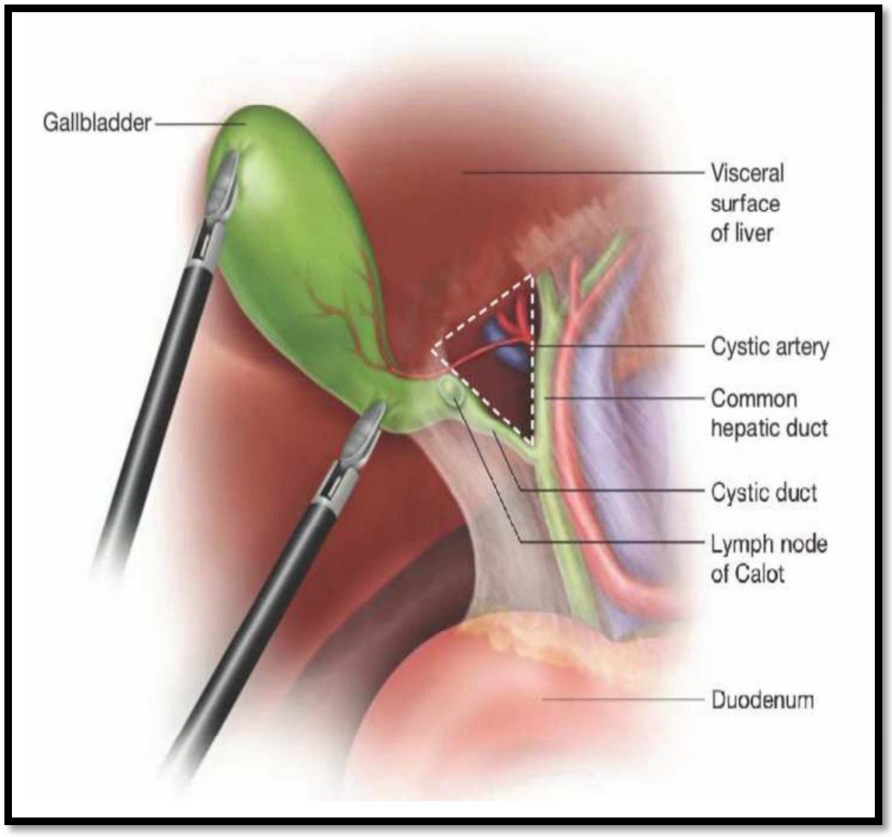


FIGURE 11 : CALOT'S TRIANGLE

BOUNDARIES:

Calot's triangle is an anatomical landmark in the vicinity of the gallbladder, is defined by the following boundaries:

- Superiorly: Segment V of inferior border of the liver
- Medially: Common hepatic duct(CHD)
- Laterally: Cystic duct and neck of the gallbladder

Contents within Calot's triangle typically include:

1. Right hepatic artery
2. Cystic artery(CA)
3. Cystic lymph node of Lund
4. Small cystic veins
5. Autonomic nerves piercing the gallbladder
6. Adipose tissue
7. Some accessory ducts draining the gallbladder.

9.LAPAROSCPOIC ANATOMY OF GALLBLADDER

In laparoscopic surgery, the initial view often involves observing the subphrenic space, the abdominal side of the diaphramg, and the diaphragmatic surface of the liver. The gallbladder fundus is typically visible on inferior surface of liver. The right and left subphrenic spaces are divided by the falciform ligament.

Rouviere’s sulcus, a distinct groove or fissure between the caudate lobes of the liver and right lobe of liver, is usually present and is visible in most individuals. It is located where the right pedicle enters the liver at the level of porta hepatis. Dissection during surgery should be conducted above this sulcus to avoid injuring the bile duct.

The concept of the "critical view of safety" is crucial in laparoscopic gallbladder surgery to avoid bile duct injury. It involves dissecting the tissues in Calot's triangle while ensuring that only the cystic artery and cystic duct are visible entering the gallbladder. This dissection technique, described by Strasberg et al., exposes the base of the liver bed and minimizes the risk of damaging the bile duct.[12]

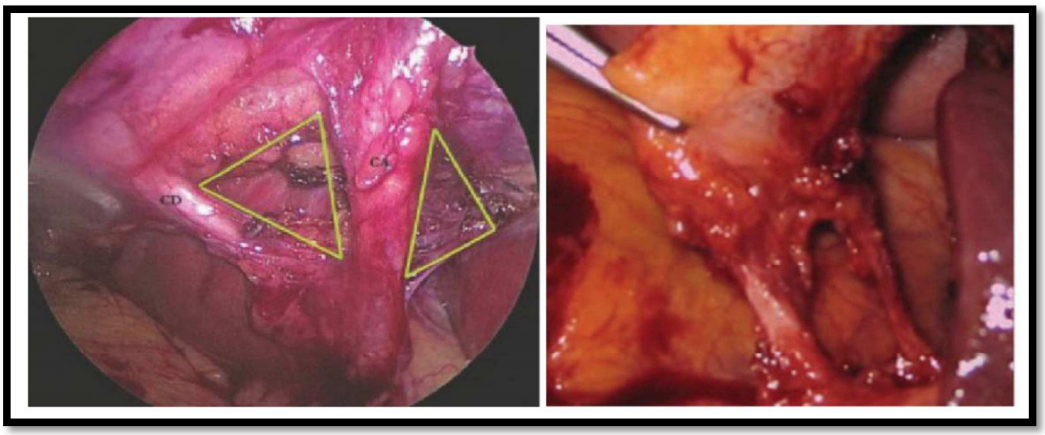


FIGURE 12(A) : Critical view of safety in laparoscopic cholecystectomy

FIGURE 12 (B) The critical view in open cholecystectomy.

C.PHYSIOLOGY OF GALLBLADDER

In a healthy adult, the liver secretes approximately 500 to 1000 milliliters of bile per day in response to various stimuli such as neural, hormonal, and chemical signals. The gallbladder serves as a cistern for bile, storing it before releasing it into the duodenum intermittently. The flow of bile into the duodenum is governed by Sphincter of the Oddi.[8]

During fasting periods, the sphincter contracts, creating a pressure difference between the bile duct and the gallbladder, which allows the gallbladder to fill. After consuming a meal, there is a coordinated relaxation and contraction of the sphincter of Oddi, facilitating the emptying of bile into the duodenum. Cholecystokinin, secreted by the duodenal mucosa in response to low pH and food consumption, plays a vital role in regulating the actions of the sphincter by acting on the smooth muscle fibers of the gallbladder.

Typically, about 95% of bile is reabsorbed into the terminal ileum through enterohepatic circulation, contributing to the efficient recycling of bile constituents.[8,9]

FUNCTIONS OF GALLBLADDER-

1. The gallbladder serves as a storage organ for bile, containing approximately 80% of the bile produced by the liver, particularly during fasting periods.
2. It concentrates hepatic bile by a factor of 5 to 10 times through the absorption of bicarbonate, water, and sodium chloride.
3. The gallbladder secretes mucus, providing mucosal protection to its lining.
4. It helps maintain pressure within the biliary tree, ensuring efficient bile flow.

Water absorption in the gallbladder occurs through both passive and active mechanisms, resulting in variations in biliary concentration between the liver and gallbladder. Additionally, the gallbladder readily absorbs calcium and magnesium.

Comparatively, gallbladder bile tends to be more acidic than hepatic bile due to the absorption of chloride, bicarbonate, and hydrogen ions, contributing to differences in composition between bile stored in the gallbladder and that produced by the liver.[9]

Characteristics*	Hepatic Bile	Gallbladder Bile
Na	160	270
K	5	10
Cl	90	15
HCO_3	45	10
Ca	4	25
Mg	2	4
Bilirubin	1.5	15
Protein	150	200
Bile acids	50	150
Phospholipids	8	40
Cholesterol	4	18
Total solids	—	125
pH	7.8	7.2

*All determinations are milliequivalents per liter; except for pH. Significant ranges of all elements may occur.

TABLE 1 : BILE COMPOSITION

BILE ACIDS AND SALT

Bile acids play a important role in the absorbtion and digestion of fats in the gut. Here are some key points about bile acids:

1. **Primary Bile Acids**: These include chenodeoxycholate and cholate . They are synthesized in the liver from cholesterol.
2. **Secondary Bile Acids**: Lithocholate and Deoxycholate are examples of 2^o bile acids. They are formed in the gut through the action of bacteria on primary bile acids.
3. **Tertiary Bile Acids**: Ursodeoxycholate is an example of a tertiary bile acid.

Bile acids aid in fat digestion and absorption by forming anionic conjugates with glycine and taurine. Approximately 500 to 600 milligrams of bile acids are produced in a day.

In the gut, approximately 80% of conjugated bile acids are absorbed in the terminal part of the ileum. The remaining bile acids, along with those deconjugated by bacteria in the colon, form 2^o bile acids.

A significant portion, about 95%, of bile acids enters the enterohepatic circulation, where they are reabsorbed in the terminal part of the ileum and returned to liver, completing the enterohepatic circulation loop.

BILE PIGMENTS

The primary Bile pigment, bilirubin, is predominantly formed from the breakdown of old red blood cells (RBCs) by the reticuloendothelial system, primarily in the spleen and liver. Biliverdin is an intermediate product in this breakdown process and is produced from bilirubin through a series of enzymatic reactions. Specifically, biliverdin is formed when bilirubin is oxidized. Eventually, biliverdin is

converted back into bilirubin before being conjugated in the liver and excreted into bile.

BILIRUBIN METABOLISM AND EXCRETION

Absolutely, the breakdown of hemoglobin results in the formation of bilirubin. Once bilirubin is released into circulation, it binds to albumin for transport. Hepatocytes, liver cells, absorb free bilirubin by binding it to cytoplasmic proteins. Inside the hepatocytes, bilirubin undergoes conjugation by an enzyme called UDP-glucuronyl transferase, which attaches glucuronic acid molecules to bilirubin, forming bilirubin diglucuronide. This conjugated bilirubin is water-soluble.

The majority of bilirubin diglucuronide is actively transported into bile canaliculi, against the concentration gradient, for excretion into bile. A small portion may also be disposed of in the urine, contributing to the yellow color of urine in cases of excess bilirubin production or impaired bilirubin excretion.

ENTEROHEPATIC CIRCULATION

Bile salts play a important role in the absorption and digestion of fats. Here are some key points about bile salts:

1. **Formation and Composition**: Bile salts are secreted by hepatocytes into canaliculi and then into small bile ducts. They contain sodium and potassium salts which conjugate with amino acids glycine and taurine, forming primary bile salts. In the intestine, bacterial flora alters primary bile salts to form 2^{o} bile salts, such as lithocholate and deoxycholate.
2. **Function**: Bile salts aid in the solubilization of lipids by forming micelles, thus facilitating their absorption in the intestines.
3. **Maintenance of Bile Salt Pool**: The bile salt pool is primarily maintained by the gallbladder, followed by the liver and small intestine.

4. **Absorption**: The small intestine absorbs about majority 90-95% of bile salts. This absorption occurs through nonionic diffusion or primarily via sodium bile salt co-transport, which involves Na^+/K^+-ATPase present in the terminal ileum.
5. **Resynthesis and Recycling**: The liver synthesizes bile salts at a rate of 0.2 to 0.4grams per day. However, about 3.5 grams of bile salts are repeatedly recycled through the enterohepatic circulation, as they are efficiently reabsorbed from the intestines and returned to liver.

D.NATURAL COURSE OF GALLSTONE DISEASE

Gallstones are prevalent, yet many individuals remain asymptomatic throughout their lives. When symptoms do arise, they often manifest as postprandial right upper abdominal pain, typically due to a calculus lodged in the cystic duct. However, gallstones can lead to various complications, including acute cholecystitis, gallstone pancreatitis, cholangitis choledocholithiasis, gallstone ileus, and gallbladder carcinoma.

Studies have examined the likelihood of symptomatic development following incidental diagnosis of gallstone disease. Approximately 80% of patients remain asymptomatic, while around 2 to 3% become symptomatic each year. Among symptomatic patients, 3 to 5% are known to develop complicated gallstone disease.

Perforation of the gallbladder frequently occurs in its fundus, which is the least vascular region. The term "white bile" describes a condition where the CD is obstructed by gallstones, leading to the backup of bile. This impaction commonly occurs in Hartmann's pouch, a dilated area of cystic duct near its junction with the gallbladder.[9]

1. EPIDEMIOLOGY

The prevalence of gallstones among Indian adults varies from 6%-20%.There are various risk factors contributing their formation. Here are some key factors:

1. **Female Sex and Pregnancy**: Women, especially during pregnancy, have a three-fold increased risk of developing gallstones. Elevated estrogen levels increase cholesterol secretion, while progesterone delays gallbladder emptying.
2. **Age**: The likelihood of gallstone formation increases with age.
3. **Obesity**: Obesity is linked with decreased gallbladder contractility due to cholesterol supersaturation, which promotes calculus formation.
4. **Rapid Weight Loss**: Rapid weight loss, often associated with calorie restriction, can lead to excess mucin release and hepatic cholesterol production, resulting in the formation of sludge.
5. **Drugs**:Certain medications, including oral contraceptive pills, estrogen therapy, third-generation cephalosporins, thiazides, and octreotide, are known to increase the risk of gallstones.
6. **Systemic Illnesses**: Insulin resistance associated with hypercholesterolemia in diabetes, reduced bile salt secretion in liver cirrhosis, and chronic hemolysis can contribute to the formation of pigmented stones.
7. **Gastrointestinal Conditions**: Conditions like Crohn's disease or terminal ileal resection can impair the absorption of bile salts, leading to bile supersaturation and stone formation.
8. **Haematological Conditions**: Hemolytic anemia, sickle cell disease, thalassemia, and hereditary spherocytosis increase the propensity for pigmented stone formation due to increased bilirubin levels.

2. FROMATION OF GALLSTONES

Gallstone formation is indeed a multifactorial process, often involving imbalances between bile acids and cholesterol concentrations in bile. Here's how it typically occurs:

1. **Supersaturated Bile**: Cholesterol is insoluble in water but is held in a stable micelle by phospholipids in bile. Supersaturation of bile occurs when there's a decrease in bile acid or lecithin secretion, leading to excess cholesterol, which can then form cholesterol calculi.
2. **Cholesterol Nucleation**: In cholesterol-saturated bile, stones can form from unilamellar phospholipid vesicles after cholesterol nucleation. Glycoproteins and mucin aid in the nucleation process. Beta-glucuronidase produced by bacteria like E. coli can hydrolyze bilirubin glucuronide, forming insoluble deconjugated bilirubin. This complex can then bind with calcium, contributing to stone formation in the biliary tree.[8]
3. **Impaired Gallbladder Function**:Presence of stones in the biliary tree can delay gallbladder emptying, leading to stasis. Absorption of calcium and secretion of glycoproteins and mucin by the mucosa can interfere with gallbladder contractility and emptying.
4. **Enterohepatic Circulation of Bile Acids**: Factors such as fecal colonic flora, ileal resection, deoxycholate levels, and the use of bile acid sequestrants like cholestyramine can affect bile acid absorption, potentially contributing to gallstone formation.

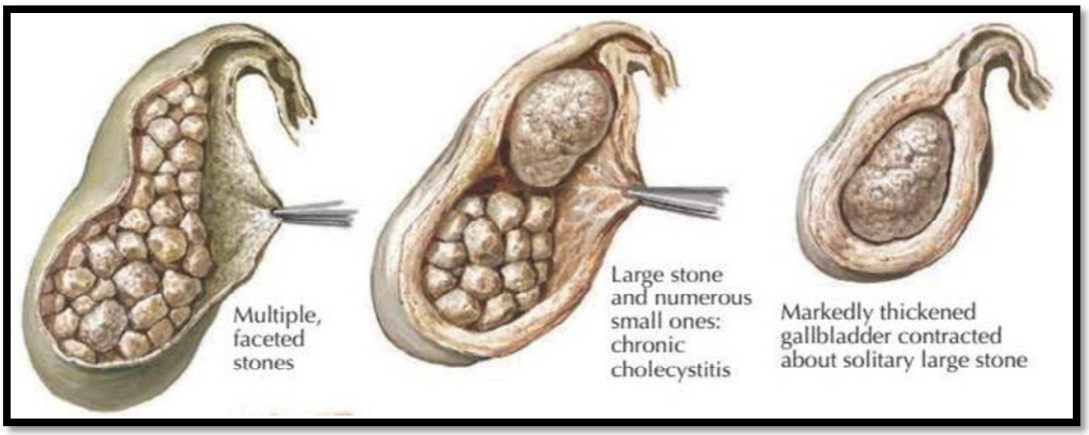


FIGURE 13 : GALL STONES

IRON- ITS ROLE IN FORMATION OF GALLSTONES

Lately the studies have underscored the importance of particles like iron, copper, calcium, zinc, & low pH in the pathogenesis of gallstones. It has been shown that a deficiency in iron can change the activity of many liver enzyme, resulting in higher cholesterol saturation in the GB & the formation of cholesterol crystals.[15]

Iron acts as a coenzyme for Nitric Oxide Synthase (NOS), the enzyme accountable for producing Nitric Oxide (NO), which is essential for maintaining gall bladder tone and relaxation. The filling and emptying of the gall bladder are controlled by both excitatory and inhibitory signals, with NO playing a crucial role in normal relaxation. When the motility of the gall bladder and the sphincter of Oddi is disrupted, biliary stasis occurs, leading to the formation of cholesterol crystals in the gall bladder.

Nitric oxide (NO) is generated from L-arginine through the activation of NO synthase (NOS), which has three isoforms produced by different genes. Two types of constitutive NOS are involved in regular physiological processes: Neuronal NOS (nNOS), primarily found in neurons[16], and Endothelial NOS (eNOS), primarily found in the endothelium[17]. Inducible NOS (iNOS), on the other hand, is not

normally present in tissues under standard physiological conditions but is produced during tissue injury and inflammation.[18]

In the gastrointestinal (GI) tract, NO has been shown to be a key inhibitory nonadrenergic, noncholinergic (NANC) neurotransmitter. NO released in response to nerve stimulation causes the relaxation of GI tract smooth muscle.[19-22]

Neuronal nitric oxide synthase (nNOS) is crucial for regulating bile flow and storage in the gallbladder. Iron-containing heme is an essential co-factor for all three isoforms of nitric oxide synthase (NOS), and without sufficient heme, NOS cannot function properly.[23-27] The deficiency of this enzyme is linked to reduced local production of NO, which leads to impaired relaxation in the GI tract.[19]

Human studies using nitric oxide-donating drugs have shown hampered emptying of gallbladder.[28] Neurons containing nNOS have been identified in gallbladders of both human being[29] and prairie dogs[30]. Therefore, any changes in biliary nNOS can lead to gallbladder stasis. Various studies have demonstrated that iron-deficient prairie dogs exhibit increased gallbladder volumes, decreased biliary ratios of specific activity (an indicator of gallbladder stasis), increased resting activity of the sphincter of Oddi, and a decreased response of the sphincter of Oddi to excitatory stimuli like cholecystokinin[31-33]. Additionally, Kaufman et al. showed that the resting tone of the sphincter of Oddi is elevated when the effective NOS inhibitor Nv-nitro-L-methyl ester (L-NAME) is administered intravenously, suggesting that NOS is crucial for normal resting tone of the sphincter of Oddi. Collectively, these studies indicate that iron deficiency, by reducing nNOS, promotes bile stasis.[23]

In similar studies done by Hamid H Sarhan et al.[34] & Kumar Muneesh et al.[35], 50 patients with ultrasonography-confirmed cholelithiasis were divided into non-anemic and anemic groups. The researchers measured serum cholesterol and

gallbladder bile cholesterol levels in both groups. They found that gallbladder bile cholesterol was considerably higher in anaemic individuals. The studies concluded that low serum iron levels result in bile supersaturation with cholesterol, which results in gallstone formation.

Abnormal gallbladder motility causes bile stasis, which provides more time for solutes to precipitate. Consequently, stone formation is often observed in conditions associated with impaired gallbladder emptying[40]. Bile stasis results in increased cholesterol saturation in the gallbladder, promoting the formation of cholesterol crystals and eventually leading to the development of cholesterol stones.

3. TYPES OF GALL STONES

Gallstones can be classified into three main types based on their composition:

1. **Cholesterol Stones**:

- Pure cholesterol stones are rare; most are mixed stones containing over 70% cholesterol along with calcium and bile pigments.
- They typically have a smooth surface and are single large stones.
- Cholesterol stones are pale yellow in color.
- The majority of them are radiolucent, meaning they do not appear on X-rays.

2. **Pigment Stones**:

- Pigment stones can be further divided into brown stones and black stones.
- Black stones: Their color is attributed to the presence of calcium bilirubinate complexes along with calcium phosphate and calcium bicarbonate. They are tiny, brittle, and spiculated in appearance. They are commonly seen in conditions such as hemolysis, liver cirrhosis, sickle cell disease and hereditary spherocytosis.
- Brown stones: These stones are semisolid and tiny. They are found in common bile duct (CBD) and rarely in gallbladder. Their composition includes, calcium

palmitate, calcium stearate, calcium bilirubinate and cholesterol. Brown stones can also be associated with parasitic infections such as Ascaris lumbricoides and Clonorchis sinensis

3. **Mixed Stones**:

- Mixed stones are characterized by a combination of constituents.
- They typically have multiple facets on their surface.
- These stones have a pure central core surrounded by an outer layer made from a mixture of cholesterol, calcium, and bile pigments.
- The color of mixed stones depends on the composition of their constituents.
- Inflammatory processes can contribute to the formation of mixed stones, which can be identified by the presence of epithelial debris and bacteria in the laminated central nucleus.

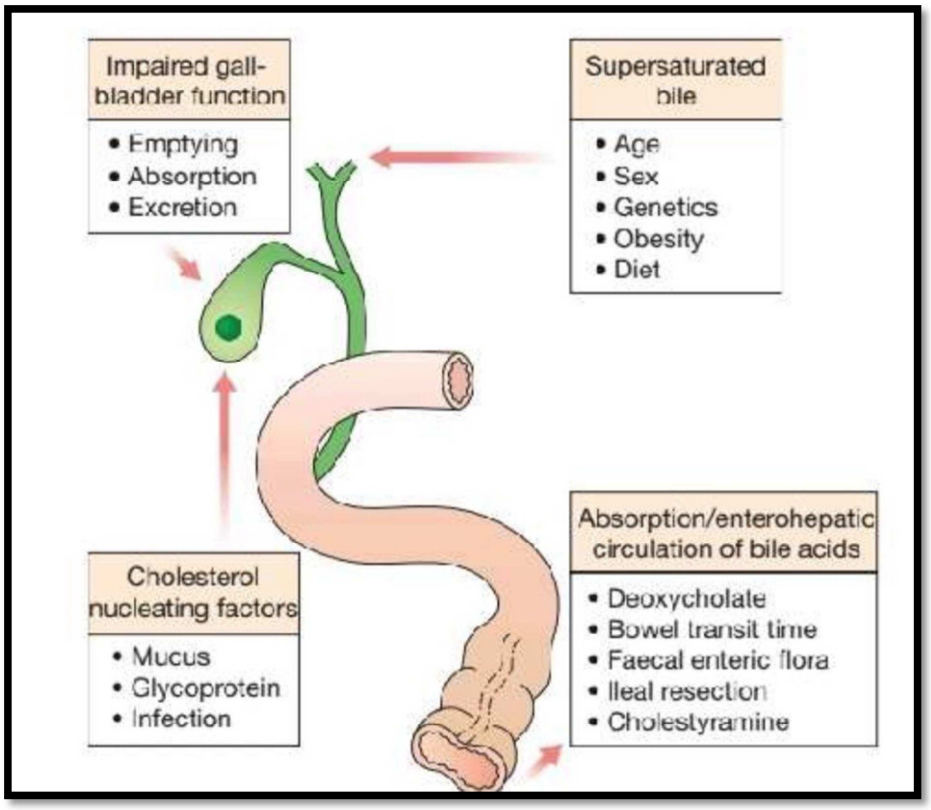


FIGURE 14 : FACTORS ASSOCIATED WITH GALLSTONE FORMATION

LAPARASCOPIC CHOLECYSTECTOMY

The timing of cholecystectomy for acute cholecystitis is indeed a topic of debate among surgeons, and there are differing opinions on when it should be performed. Here are some key points to consider:

1. **Early Intervention:** Many surgeons advocate for early cholecystectomy, typically within 5 to 7 days of the onset of symptoms, especially in patients with uncomplicated acute cholecystitis. Early surgery has been associated with shorter hospital stays leading to decreased risk of complications, and early recovery.
2. **Optimal Timing:** However, the optimal timing of surgery varies depending on the severity of the patient's condition, the presence of complications, and individual patient factors. Some surgeons may prefer to delay surgery until the acute inflammatory process has subsided, typically around 6 weeks after the initial attack, to reduce the risk of complications and improve surgical outcomes.
3. **Experience and Facilities:** The success of early cholecystectomy relies on the experience of the surgeon and the availability of adequate hospital facilities. Surgeons with extensive experience in laparoscopic cholecystectomy and access to advanced surgical equipment and support staff are better equipped to perform early surgery safely and effectively.
4. **Conversion Rate:** It's important to note that the conversion rate from laparoscopic to open cholecystectomy is generally higher in cases of acute cholecystitis compared to elective cases. This is often due to factors such as inflammation, adhesions, and difficulty visualizing anatomical structures. Surgeons should be prepared to convert to an open procedure if necessary to ensure patient safety.

Ultimately, the decision regarding the timing of cholecystectomy should be individualized based on careful consideration of the patient's clinical presentation, surgical risk factors, and institutional resources. Close collaboration between the surgical team, gastroenterologists, and other healthcare providers is essential to ensure optimal patient outcomes.

STEPS OF LAPAROSCOPIC CHOLECYSTECTOMY

Here's a breakdown of the steps involved in the laparoscopic cholecystectomy procedure:

1. **Patient Positioning:** The patient is placed comfortably in a supine position on the operating table with both arms by their side. The feet are strapped to secure the patient's position and prevent movement during the procedure.
2. **Anesthesia:** General anesthesia is administered to the patient to ensure unconsciousness and pain control throughout the surgery.
3. **Intubation and Tube Insertion:** After induction of anesthesia, endotracheal intubation is performed to secure the airway. Additionally, a Foley catheter is inserted into the bladder to drain urine, and a Ryles tube (nasogastric tube) is inserted into the stomach for decompression.
4. **Pneumoperitoneum:** Pneumoperitoneum, the inflation of the abdominal cavity with carbon dioxide (CO2), is necessary to create a working space for the laparoscopic instruments. There are two techniques for creating pneumoperitoneum: [13]

Veress Needle (Closed Technique): A Veress needle is inserted through the anterior abdominal wall near the umbilicus, and its position is confirmed by aspirating normal

saline using a syringe. CO2 gas is then insufflated into the abdominal cavity, creating uniform distension.

Open Hassan's Technique: An incision is made supraumbilically, and the peritoneum is accessed directly. A trocar is then inserted into the peritoneal cavity under direct vision, and Carbon-di-oxide insufflation is performed.

5. **Placement of Ports:** Ports are inserted into the abdominal wall to provide access for the laparoscopic instruments. Typically, a 5 or 10mm port is initially placed in the periumbilical region. Additional ports are then placed under direct vision, including ports in the right midclavicular line, right anterior axillary line, and right upper quadrant.

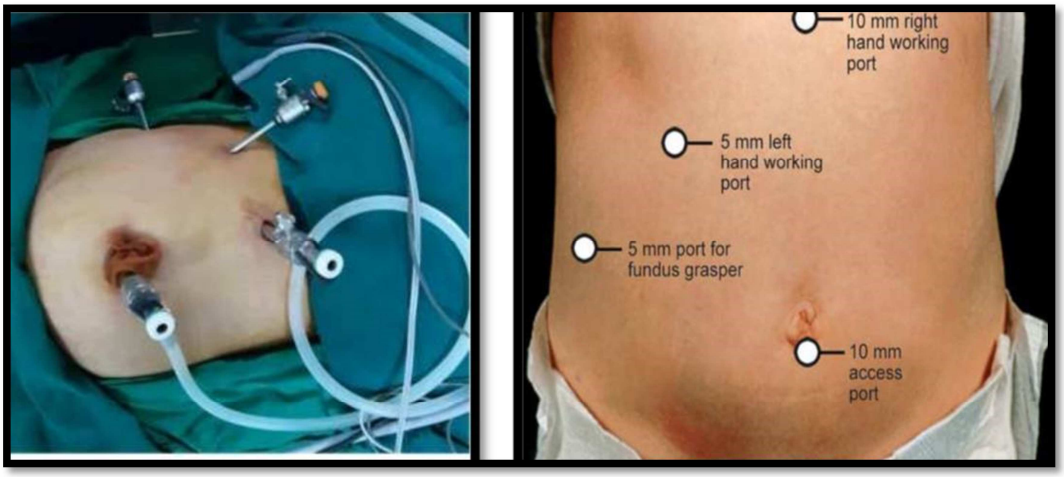


FIGURE 15: LAPAROSCOPIC PORT PLACEMENT FOR CHOLECYSTECTOMY

6. **Exposure and Inspection:** Once the ports are in place, a laparoscope (0 or 30 degrees) is inserted through one of the ports to visualize the abdominal cavity. The surgeon inspects the gallbladder, liver, omentum, bowel, and pelvis for any abnormalities or adhesions.

7. **Positioning:** The patient is placed in a reverse Trendelenburg position with a left tilt of 15 degrees. This positioning helps to move the colon and omentum away from the surgical site, providing better exposure and access to the gallbladder.

These steps ensure proper preparation and positioning of the patient and provide the surgeon with adequate visualization and access to perform the laparoscopic cholecystectomy safely and effectively.

Here's a summary of the remaining steps in the laparoscopic cholecystectomy procedure:

B. **Porta Hepatis Exposure:**

The fundus of the gallbladder is lifted and retracted upwards towards the right shoulder using an atraumatic grasper. This maneuver provides a clear view of Calot's triangle and the liver for dissection.

Any adhesions between the liver and gallbladder are carefully separated from fundus towards the neck of gallbladder using blunt dissection and cautery.

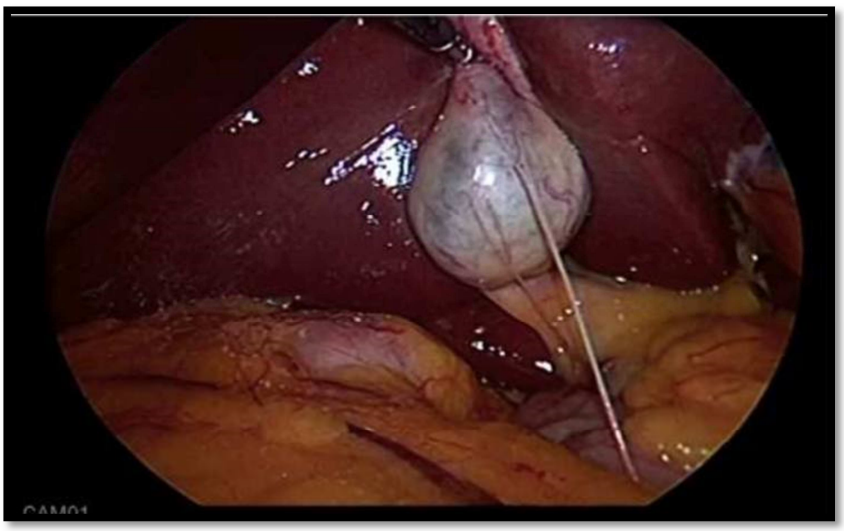


FIGURE 16 : PORTA HEPATIS EXPOSURE

C. **Calot's Triangle Dissection:**

After retraction of the infundibulum laterally for better visualization, the peritoneal layer over the neck of the gallbladder is exposed and opened using cautery.

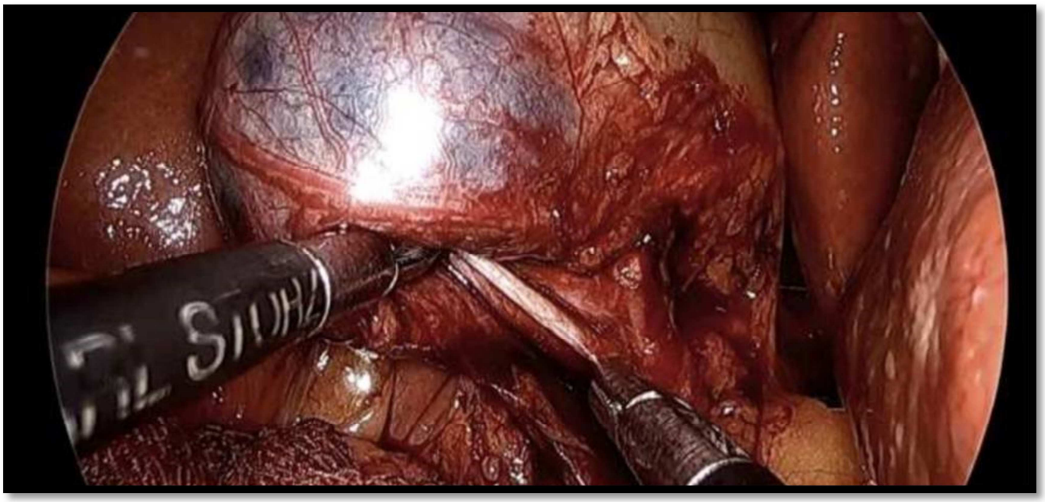


FIGURE 17: CALOT'S TRIANGLE DISSECTION

D. **Obtaining Critical View of Safety:[14]**

Complete dissection of Calot's triangle is crucial to prevent inadvertent bile duct injuries. Only two structures, the cystic artery and cystic duct, should be seen passing to the gallbladder.

The cystic duct is ligated with two clips on the duct side and one over the gallbladder side. Any stones present in the cystic duct are milked towards the gallbladder before application of clips. The cystic artery is ligated before the cystic duct.

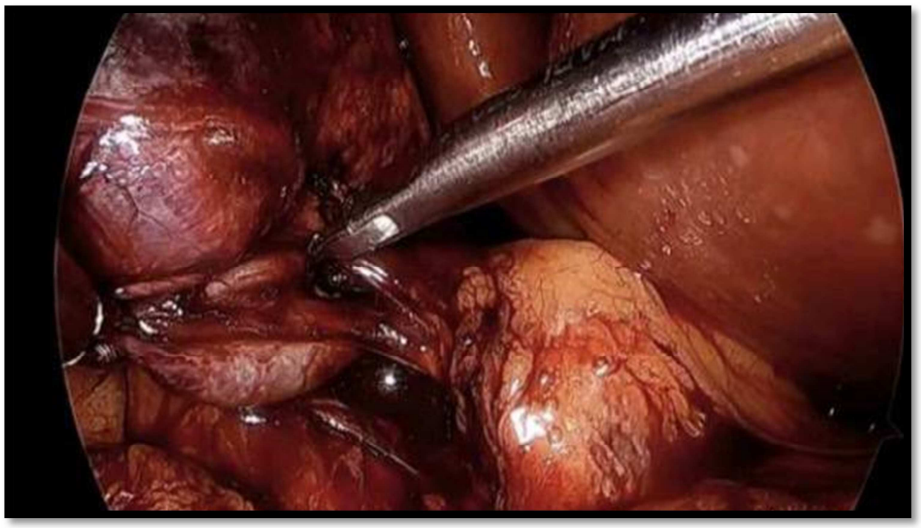


FIGURE 18: CLIPPING OF CYSTIC ARTERY

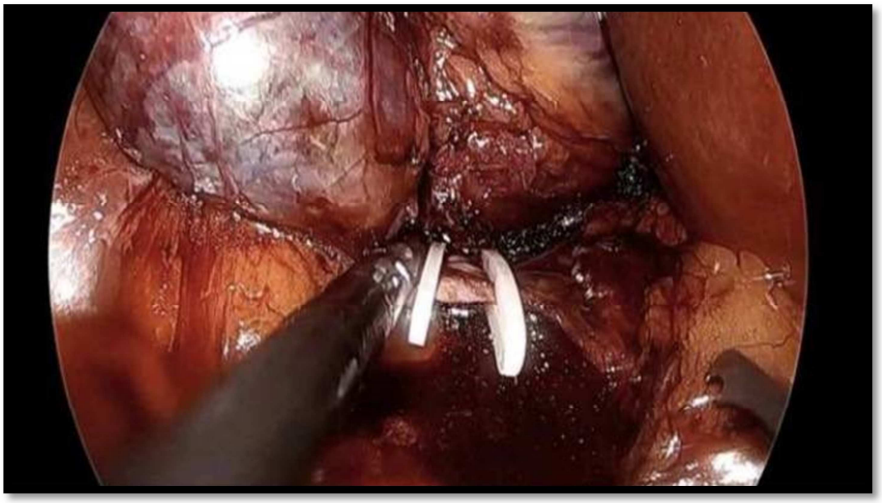


FIGURE 19: CLIPPING OF CYSTIC DUCT

E. **Detachment of Gallbladder from Fossa:**

The cystic plate is exposed after dissecting the gallbladder fossa from its bed using monopolar hook cautery.

If there is iatrogenic spillage of bile due to gallbladder perforation, it is controlled by tying or grasping. Immediate irrigation and suction of bile are performed, followed by confirmation of hemostasis and checking the position of clips.

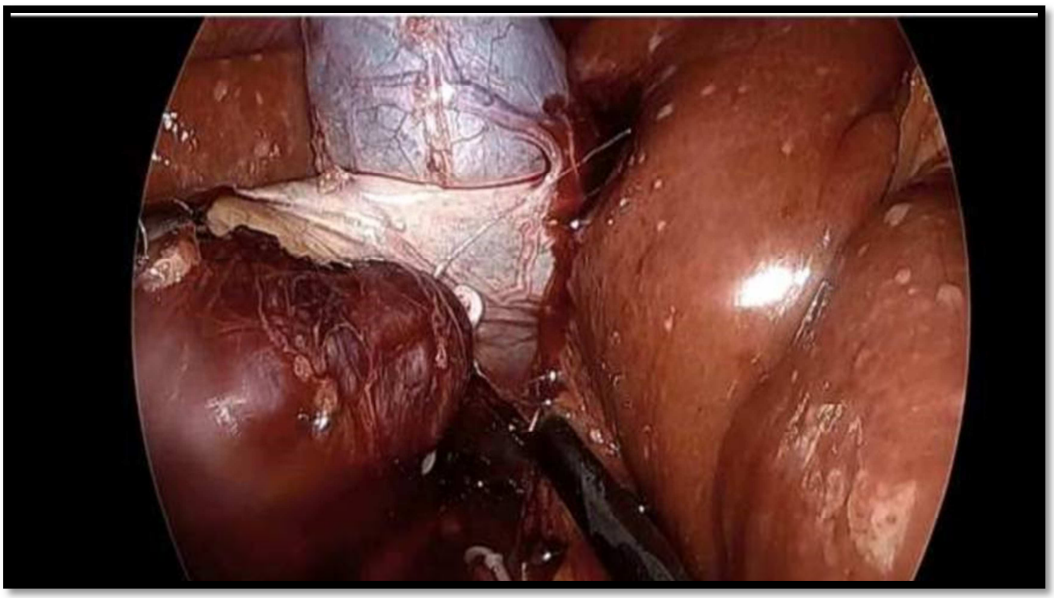


FIGURE 20 : DETACHING GALL BLADDER FROM FOSSA

F. **Extraction of Gallbladder Specimen:**

The camera is placed in the subxiphoid port, and the gallbladder, placed in an endobag, is removed through the umbilical port.

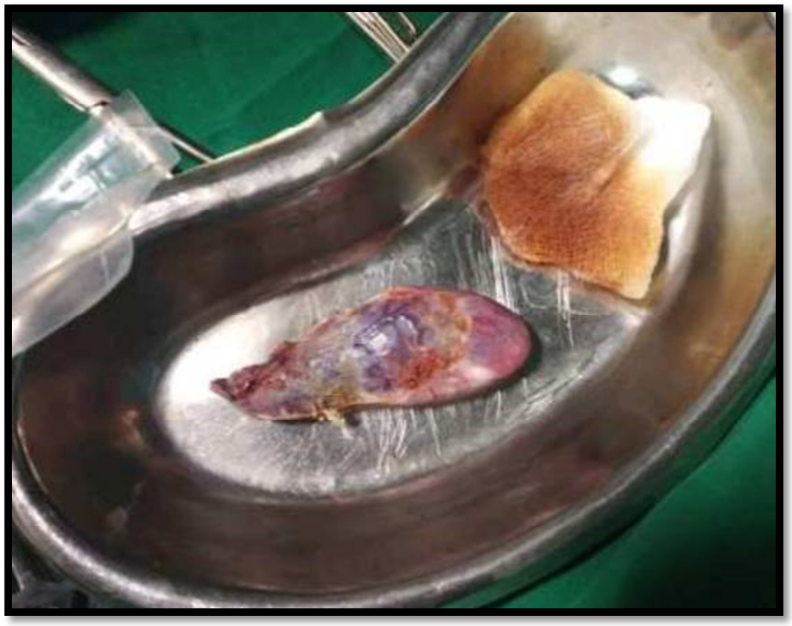


FIGURE 21: GALLBLADDER SPECIMEN AFTER EXTRACTION

G. **Inspection and Irrigation:**

After gallbladder extraction, the ports are reinserted to inspect for any bleeding. Thorough saline wash is performed in the gallbladder fossa and paracolic gutter.

H. **Drainage and Closure:**

A drain is placed if there is suspected bleeding from the liver bed or iatrogenic bile spillage through gallbladder perforation.

METHODOLOGY

This was a cross sectional observational study conducted in Department of General Surgery in KLES Dr Prabhakar Kore Hospital and Medical Research Centre, KAHER, Belagavi for duration of one year from September 2022 to August 2023

Source of Data: Patients diagnosed with gallstone disease by CT scan or Ultrasonography on outpatient department and emergency medical department KLES Dr Prabhakar Kore Hospital & MRC, KAHER Belgavi.

Inclusion Criteria:

- All cases diagnosed with Cholelithiasis
- Age between 15-90 years of age

Exclusion Criteria

- Patients who have undergone a biliary tract surgery
- Patients taking iron supplements.

Study Design- A cross-sectional observational study

Sample Size- 85

Sampling Procedure-

The minimum sample size formula based on prevalence rate is

$$n=\frac{z_{\alpha}{}^{2}P(1-P)}{d^{2}}$$

where P is the prevalence rate and d is the percentage likely difference in the prevalence.

z α is linked with the level of significance. For 5% level of the significance z α = 1.96.

The parameter considered in the calculation is the prevalence rate of low serum iron in males which is 41.93%

With P = 41.93% and d = 25% of P = 10.48%, **the sample size is 85.**

Statistical Analysis

Procedure:

- Patients complaining of epigastric pain, vomiting admitted in Department of General Surgery ward in KLES Dr Prabhakar Kore Hospital & MRC ,undergoing ultrasonography or CT scan ,diagnosed with cholelithiasis, pre-operatively serum iron and serum ferritin was sent along with other routine investigations to check the severity of anaemia with cholelithiasis
- As per the laboraroty reports normal serum iron levels in in both males and females is 33-193 ug/dl and serum ferritin levels are 30-400 ug/dl
- The normal haemoglobin levels in males is 13.0-16.0 mg/dl and in females it is 12.0-15.0 mg/dl
- Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. Non normally distributed quantitative variables were summarized by median and interquartile range (IQR). Data was also represented using appropriate diagrams like bar diagram, pie diagram and box plots.
- All Quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal

distribution. Shapiro wilk test p value of >0.05 was considered as normal distribution.

- Categorical outcomes were compared between study groups using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.)
- P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.(41)

RESULTS

- A study of 85 patients of cholelithiasis admitted in KLEs Dr Prabhakar Kore Hospital & MRC Belagavi.
- This is a Cross sectional observational study done to asses the co-relation between serum iron and serum ferritin as a parameter for gall stone disease.
- The Results were analysed from the observations made and are tabulated as follows:

Table 2: Descriptive analysis of Age in (years) in study population (N=85)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Age	51.96 ± 14.85	54.0	18.0	82.0	48.8	55.2

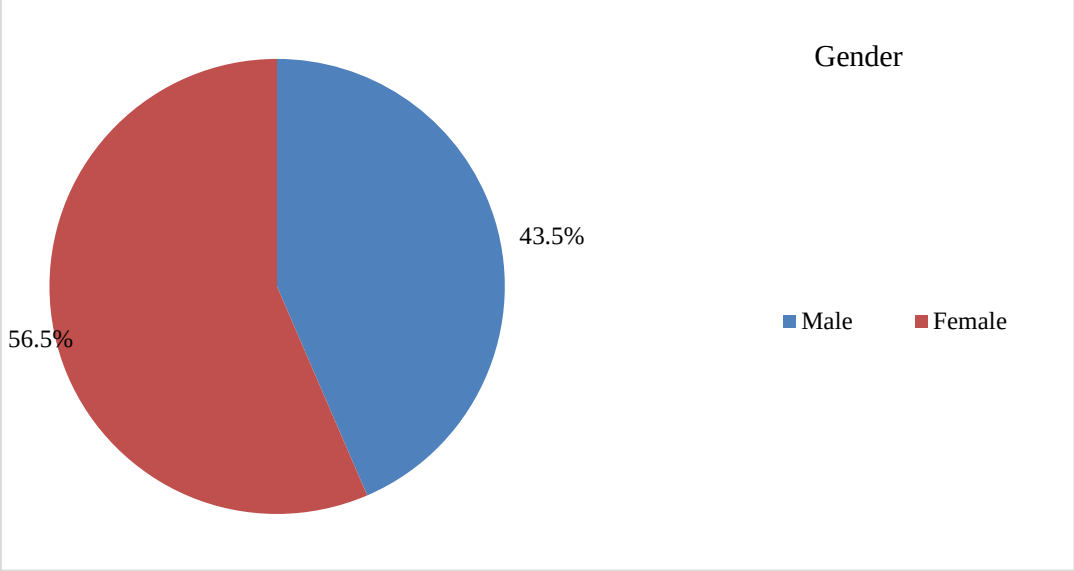
- The average age of the population was 51.96 years with standard deviation of 14.85 years
- The middle value when the ages are ordered from youngest to oldest is 54 years with youngest age of 18 years and oldest of 82 years.
- 95% Confidence Index that the true mean age of the population lies within 48.8 years to 55.2 years

Table 3: Descriptive analysis of gender in the study population (N=85)

Gender	Frequency	Percentages
Male	37	43.53%
Female	48	56.47%

The Gender distribution within the study population is as follows

- There are 37 males and 48 females in the study population which comprise of 43.53%and 56.47% of total study population
- These visual representation clearly depict that the study population have a higher representation of females as compared to males



Pie chart of gender in the study population

Table 4: Descriptive analysis of Cholelithiasis in the study population (N=85)

Cholelithiasis	Frequency	Percentages
Present	85	100%

- All 85 participants(100%) have been diagnosed with cholelithiasis
- This indicates that every Indiviual in study population has cholelithiasis, providing a focused group for studying the condition. The data reflects complete prevalence of cholelithiasis in the population under study

Table 5: Descriptive analysis of serum iron, serum ferritin and haemoglobin in study population (N=85)

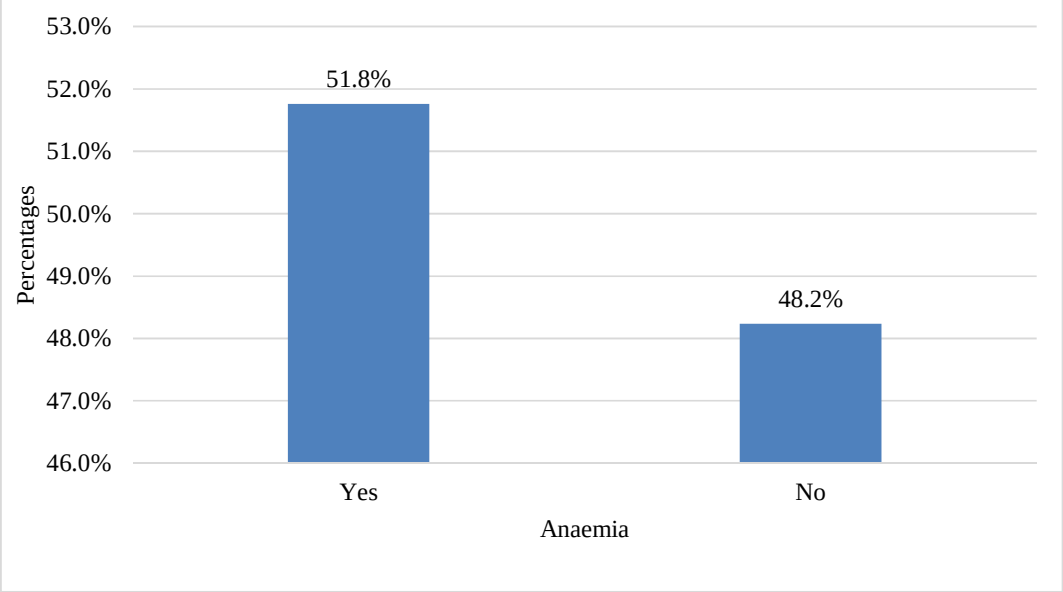
Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Serum Iron	46.67 ± 34.61	37.0	10.0	248.0	39.2	54.1
Serum Ferritin	213.46 ± 345.67	103.0	4.7	2458.0	138.9	288.0
Haemoglobin	11.95 ± 2	12.0	6.7	16.6	11.5	12.4

- The average serum iron is 46.67ug/dl with standard deviation of 34.61ug/dl.
- The median Serum iron value is 37.0 ug/dl
- The range of serum iron from minimum of 10.0 ug/dl to maximum of 248.0 ug/dl
- The true mean Serum iron level for the population is likely between 39.2 ug/dl to 54.1 ug/dl
- The average serum ferritin is 213.46 ug/dl with standard deviation of 345.67 ug/dl.
- The median Serum ferritin levels are 103.0 ug/dl.
- The range of serum ferritin from minimum of 4.7 ug/dl to maximum of 2458.0 ug/dl
- The true mean Serum ferritin level for the population is likely between 138.9 ug/dl to 288.0 ug/dl
- The average haemoglobin is 11.95 mg/dl with standard deviation of 2.0 mg/dl.
- The median haemoglobin levels are 12.0 mg/dl.
- The range of haemoglobin from minimum of 6.7 mg/dl to maximum of 16.6 mg/dl
- The true mean haemoglobin levels for the population is likely between 11.5 mg/dl to 12.4 mg/dl

Table 6: Descriptive analysis of anaemia in the study population (N=85)

Anaemia	Frequency	Percentages
Present	44	51.76%
Absent	41	48.24%

- Out of the 85 participants 44 Indiviuals(51.76%) are diagnosed with anaemia and 41 indiviuals (48.24)do not have anaemia
- This indicates that slightly more than half of the study population is affected by anaemia, making it a common condition within this group.

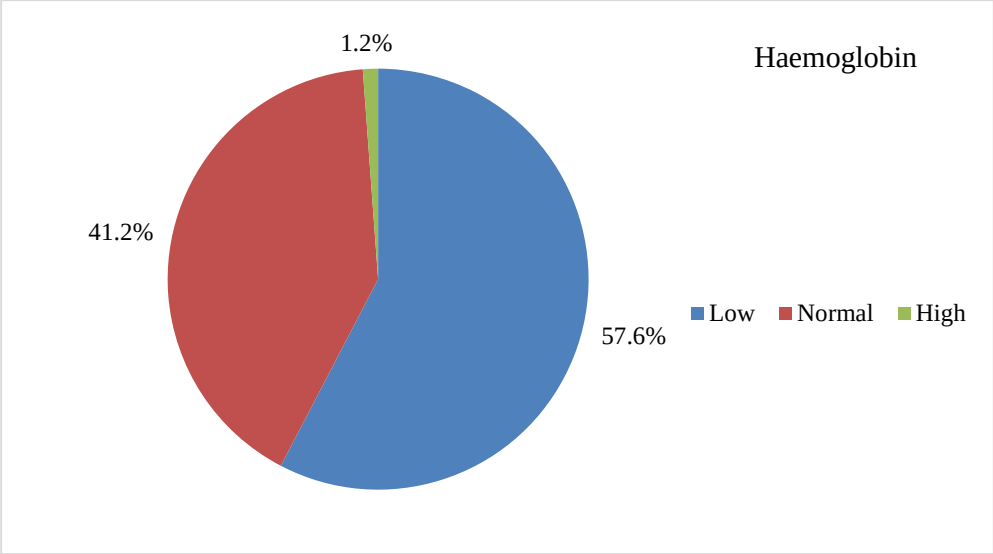


Bar chart of anaemia in the study population

Table 7: Descriptive analysis of haemoglobin, serum iron and serum ferritin in the study population (N=85)

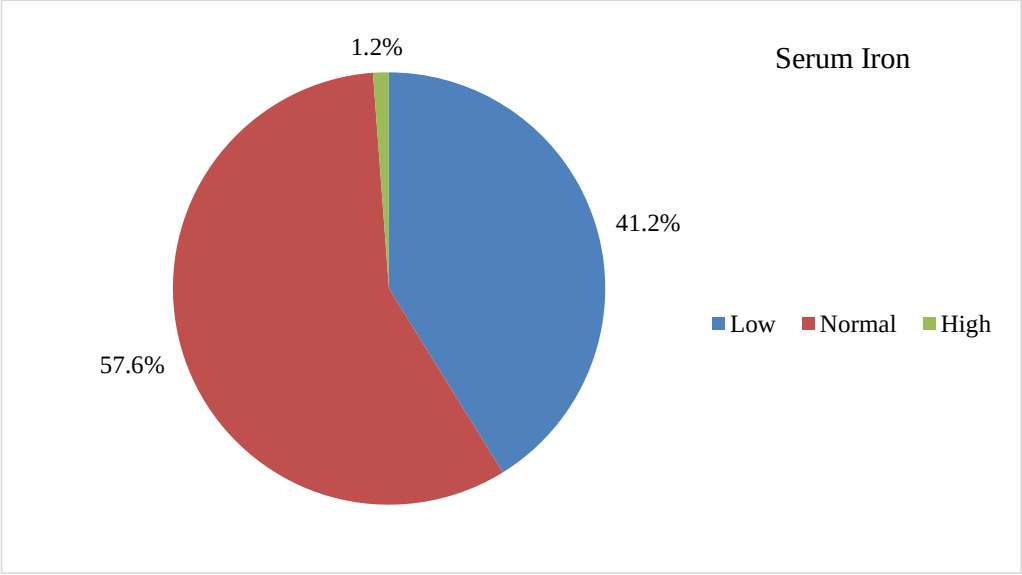
Parameter	Frequency	Percentages
Haemoglobin		
Low	49	57.65%
Normal	35	41.18%
High	1	1.18%
Serum Iron		
Low	35	41.18%
Normal	49	57.65%
High	1	1.18%
Serum Ferritin		
Low	10	11.76%
Normal	61	71.76%
High	14	16.47%

- Out of total study population (85), 49 individuals (57.65%) have low haemoglobin levels, 35 individuals (41.18%) have normal haemoglobin levels and 1 individual (1.18%) has high haemoglobin levels.
- A majority of the study population has low haemoglobin levels, indicating a potential concern for anaemia.



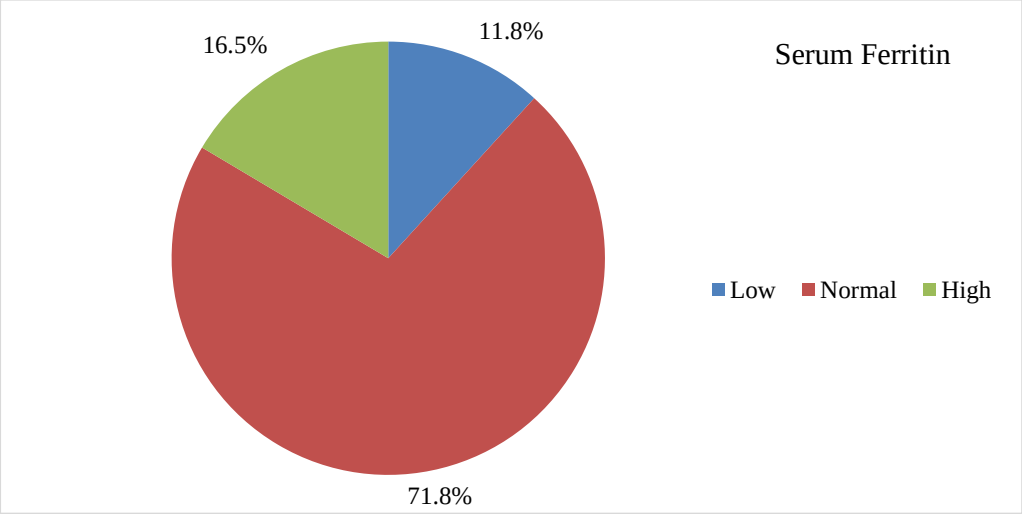
Pie chart of haemoglobin in the study population

- Out of total study population (85), 35 individuals (41.18%) have low serum iron levels, 49 individuals (57.65%) have normal serum iron levels and 1 individual (1.18%) has high serum iron levels.
- Most participants have normal serum iron levels, with a significant portion having low levels.



Pie chart of Serum Iron in the study population

- Out of total study population(85), 10 individuals (11.76%) have low serum ferritin levels, 61 individuals (71.76%) have normal serum ferritin levels, 14 individuals (16.47%) have high serum ferritin levels.
- The majority of the population has normal serum ferritin levels, with fewer individuals showing low or high levels.

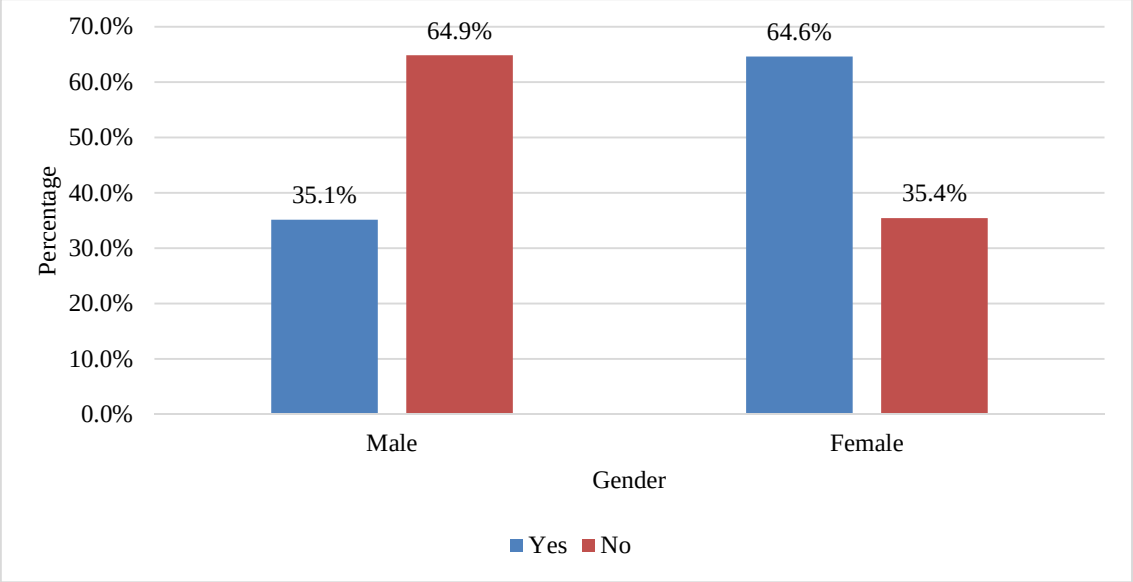


Pie chart of Serum Ferritin in the study population

Table 8: Comparison of anaemia between gender (N=85)

Anaemia	Male (N=37)	Female (N=48)	Chi Square	P value
Present	13 (35.14%)	31 (64.58%)	7.257	0.007
Absent	24 (64.86%)	17 (35.42%)		

- Out of total male study population 13 male(35.14%) have anaemia and out of total female study population 31 (64.58%) have anaemia.
- The chi-square test result (7.257) and the p-value (0.007) indicate a statistically significant association between gender and the presence of anaemia. Females have a significantly higher percentage of anaemia compared to males.



Cluster bar chart of comparison of anaemia between gender

Table 9: Comparison of mean of age between gender(N=85)

Parameter	Gender (Mean± SD)		P value
	Male (N=37)	Female (N=48)	
Age	54.84 ± 12.67	49.75 ± 16.11	0.118
Serum Iron	57.05 ± 44.87	38.67 ± 21.17	0.014
Serum Ferritin	292.12 ± 469.1	152.83 ± 190.72	0.065
Haemoglobin	12.91 ± 1.95	11.22 ± 1.73	<0.001

- Males have a slightly higher average age compared to females.
- The p-value of 0.118 suggests that this difference is not statistically significant.
- Males have a higher average serum iron level compared to females.
- The p-value of 0.014 indicates that this difference is statistically significant.
- Males have a higher average serum ferritin level compared to females
- The p-value of 0.065 suggests that this difference is not statistically significant, but it is close to the threshold of 0.05, indicating a potential trend.
- Males have a higher average haemoglobin level compared to females.
- The p-value of <0.001 indicates that this difference is highly statistically significant.

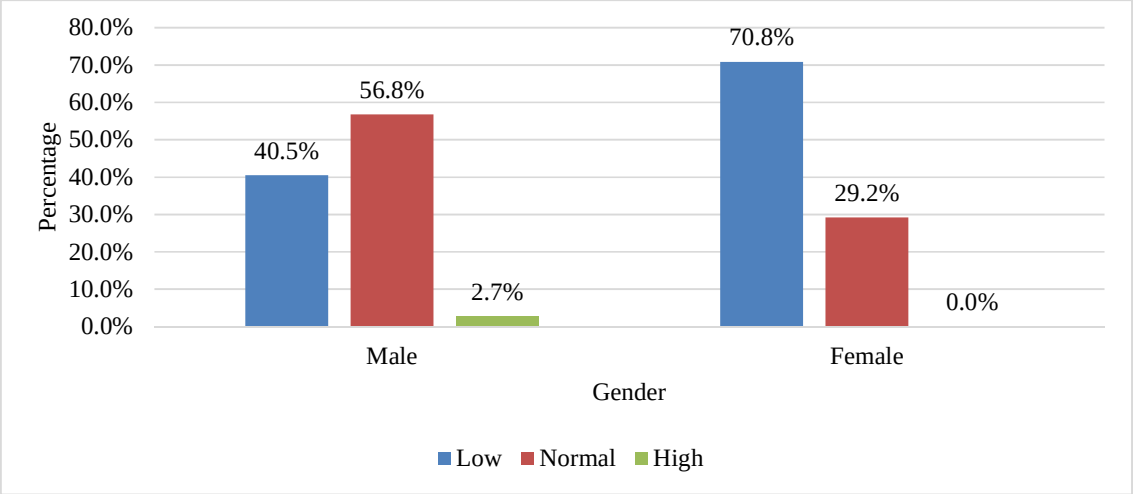
To Summarize the above data

- **Age**: No significant difference between males and females.
- **Serum Iron**: Males have significantly higher serum iron levels.
- **Serum Ferritin**: Males have higher serum ferritin levels, but the difference is not statistically significant.
- **Haemoglobin**: Males have significantly higher haemoglobin levels.

Table 10: Comparison of haemoglobin, serum iron and serum Ferritin between gender (N=85)

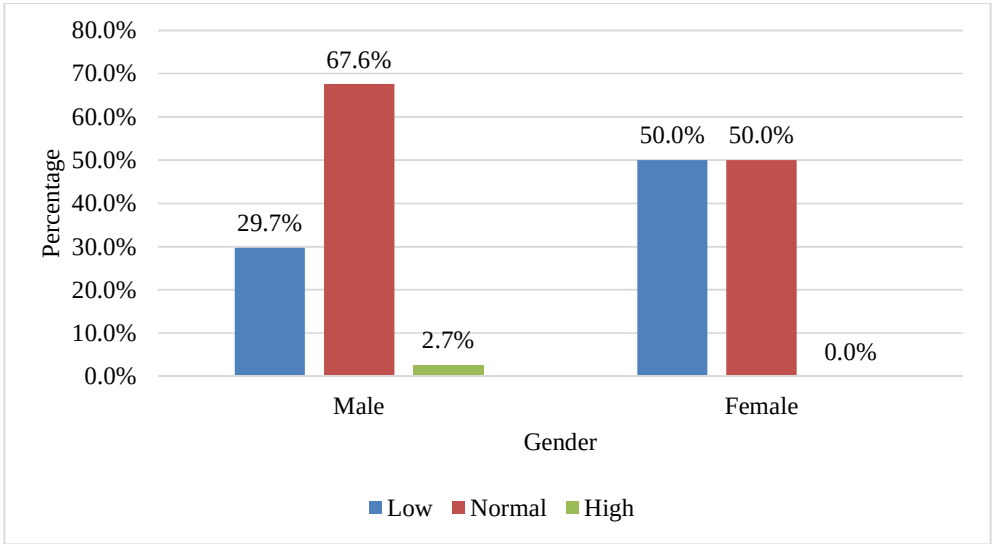
Parameter	Gender		Chi square	P value
	Male (N=37)	Female (N=48)		
Haemoglobin				
Low	15 (40.54%)	34 (70.83%)	8.486	0.014
Normal	21 (56.76%)	14 (29.17%)		
High	1 (2.7%)	0 (0%)		
Serum Iron				
Low	11 (29.73%)	24 (50%)	4.925	0.042
Normal	25 (67.57%)	24 (50%)		
High	1 (2.7%)	0 (0%)		
Serum Ferritin				
Low	2 (5.41%)	8 (16.67%)	4.826	0.044
Normal	26 (70.27%)	35 (72.92%)		
High	9 (24.32%)	5 (10.42%)		

- In the study population for Haemoglobin, a significantly higher proportion of females (70.83%) have low haemoglobin levels compared to males (40.54%).
- More males (56.76%) have normal haemoglobin levels compared to females (29.17%).
- One male (2.7%) has high haemoglobin levels, while no females have high levels.
- The chi-square value (8.486) and p-value (0.014) indicate a statistically significant difference in haemoglobin levels between genders.



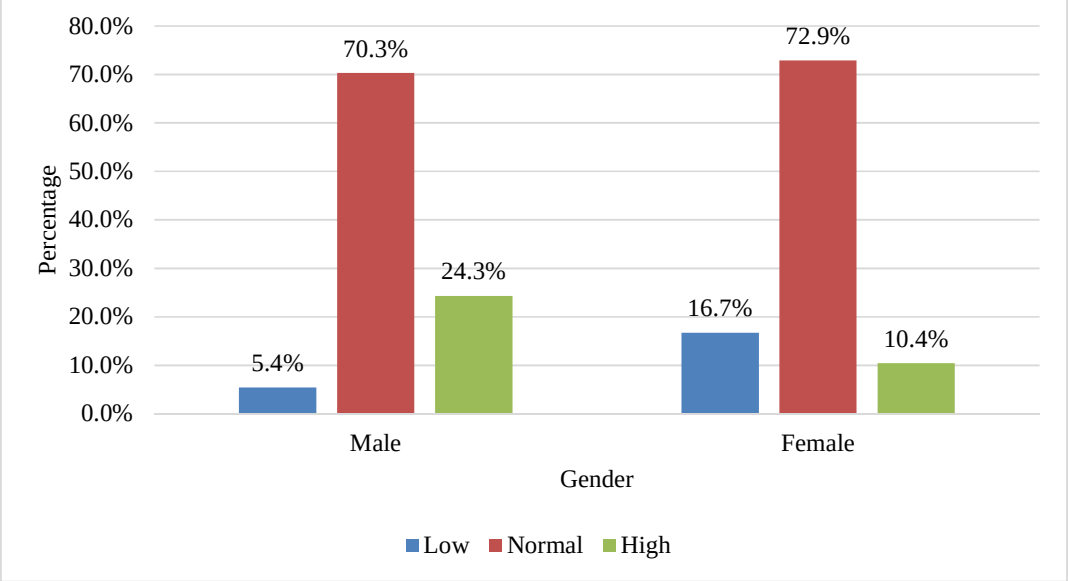
Cluster bar chart of comparison of haemoglobin between gender

- In the study population for Serum Iron, a higher proportion of females (50%) have low serum iron levels compared to males (29.73%).
- More males (67.57%) have normal serum iron levels compared to females (50%).
- One male (2.7%) has high serum iron levels, while no females have high levels.
- The chi-square value (4.925) and p-value (0.042) indicate a statistically significant difference in serum iron levels between genders.



Cluster bar chart of comparison of Serum Iron between gender

- In the study population for Serum Ferritin, a higher proportion of females (16.67%) have low serum ferritin levels compared to males (5.41%)
- Almost Similar proportions of males (70.27%) and females (72.92%) have normal serum ferritin levels.
- More males (24.32%) have high serum ferritin levels compared to females (10.42%).
- The chi-square value (4.826) and p-value (0.044) indicate a statistically significant difference in serum ferritin levels between genders.



Cluster bar chart of comparison of serum ferritin between gender

<u>To Summarize the above data</u>

- **Haemoglobin**: Significant difference, with more females having low haemoglobin levels.
- **Serum Iron**: Significant difference, with more females having low serum iron levels.
- **Serum Ferritin**: Significant difference, with more females having low serum ferritin levels.

DISCUSSION

- Cholelithiasis is a common abdominal condition which results in in patient admission and laparoscopic cholecystectomy. Cholelithiasis accounts for 10-12% of total adult population in India[1].The aim of the study is to co-relate serum iron , serum ferritin and haemoglobin as a indicator for anaemia with cholelithiasis.
- In a country like India, the prevalence of anaemia is high in mostly female population.53% of female population suffer from anaemia[7] because of blood loss during menstrual cycle, pregnancy, delivery, post-partum. With this study we can establish that iron-deficiency anaemia is one of the etiology for gallstone disease and by spreading the awareness regarding iron deficiency anaemia and need for iron supplements the incidence of gallstone disease can be reduced.

GENDER DISTRIBUTION

- Both males and females were included in the study, but the female population was majority which comprised of 56.47% of total sample population and males were 43.53% of total sample population.
- In the similar studies carried out by Arora BK et al[1], Kannan R et al[37],Dube BA et al[3], have shown majority of female group of population.

SERUM IRON LEVEL IN STUDY GROUP OF POPULATION

- As shown in Table 7, 41.18% of total sample population contained low serum iron levels in this study.
- As shown in Table 10 , Seperate serum iron has been calculated containing gender of the participants. Study showed that 29.73% of total male population and 50% of total female population had low serum iron values.

- Similar studies done by Arora BK et al[1] and DUBE BA et al[3], have calculated serum iron values in their studies using ferrozine kits. Their studies showed that lower serum iron values were more in female population compared to male population.

SERUM FERRITIN LEVELS IN STUDY GROUP OF POPULATION

- As shown in Table 7, 11.76% of total sample population contained low serum ferritin levels in this study.
- As shown in Table 10 ,serum ferritin have been calculated containing gender of the patient. Study showed that 5.41% of total male population and 16.67% of total female population had low serum ferritin values.
- Similar studies carried out by Patil G et al[6] 30% of total sample population had lower ferritin levels.
- Studies carried out by Sanjeev Kumar et al[2] showed that 42.9% of males had lower serum ferritin levels and 38.0% females have lower serum ferritin levels. Similar studies carried out by Dube BA et al[3] 33.3% of males had lower serum ferritin levels and 37% of females have lower serum ferritin levels

HEMOGLOBIN LEVELS IN STUDY GROUP OF POPULATION

- As shown in Table 7, 57.65% of total sample population contained low haemoglobin levels in this study.
- As shown in Table 10, haemoglobin have been calculated containing gender of the patient. Study showed that 40.54% of total male population and 70.83% of total female population had low haemoglobin values.

- Similar studies carried out by Naik S et al[39],71.8% females and 50% males were found to have low haemoglobin values of total sample population who suffered with gall-stone disease.

COMPARISON OF SERUM IRON,SERUM FERRITIN AND HAEMOGLOBIN AMONGST GENDERS IN STUDY GROUP OF POPULATION

- **Haemoglobin**: Significant difference was noted, with more females having low haemoglobin levels.
- **Serum Iron**: Significant difference was noted, with more females having low serum iron levels.
- **Serum Ferritin**: Significant difference was noted, with more females having low serum ferritin levels.
- Similar studies carried out by Arora BK et al[1], the mean serum iron in female study population was statistically significant and mean serum ferritin levels in female study population was statistically significant.

CONCLUSION

- In the clinical observational study, Serum iron and Serum ferritin evaluation in patients with cholelithiasis is necessary to check the severity of anaemia and its presence in cholelithiasis.
- Iron acts as important co-enzyme in bile reactions and its deficiency leads to formation of gall stones.
- Patients suffering with low iron and ferritin levels and who are yet to diagnosed with gall stone disease, iron supplementation can be provided to re-store iron levels in body and to reduce the burden of gall stone disease.
- Haemoglobin levels can also be used as indicator for Iron deficiency anaemia and appropriate intervention to correct haemoglobin levels within normal range can prevent gall stone disease.

SUMMARY

- Cholelithiasis or Gall stone disease accounts for a significant burden on health care system as it accounts for 10-15% of total population. Patients usually present with pain and dyspepsia. Ignoring gall stone disease can further lead to more severe complications and intensity of complications raises in people who suffer from other co-morbidities such as Type II Diabetes Mellitus, Ischaemic Heart disease etc. Gall stone disease can cause acute calculous cholecystitis or empyema gall bladder in patients suffering with Type II diabetes mellitus.
- Surgical intervention in patients who come with complications such as acute cholecystits or empyema gall bladder is challenging because of dense adhesions and dissection of calot's triangle.
- It is seen in our study , females population is more commonly affected with gall stone disease. It was further seen that affected population,41% of total population had lower serum iron values which was statistically significant. Female population was more affected than male population and it was proven that lower serum iron values are statistically more significant in females compared to males
- It was also seen in this study that Serum ferritin levels were low in 12% of total sample population. It was further seen that lower serum ferritin were statistically significant in female population and not significant in male population.
- Iron supplementation specially in females in reproductive age group, pregnancy, post partum can help in re-storing the iron depletion and prevent formation of gall stone disease.

LIMITATIONS

1. Smaller sample size
2. Single institute study
3. Comparison with control group study
4. Single trace element study

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ANNEXURE - I- CONSENT

INFORMED CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Objective: To relate the association of anaemia with cholelithiasis

Introduction: The old saying goes with the typical gall stone sufferer is fat, fertile, female of fifty is partly true as the disease is found in women soon after their first delivery and also in underweight and poorly nourished people Gall stone disease is a common clinical entity affecting the adult population of both sexes Cholelithiasis is a common abdominal disorder with prevalence of 10-12% in adult population and prevalence of common bile duct stones is 8-16%

Explanation of procedure:

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study. **Authorization for publication of aggregated data:** Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact: Dr _____. If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **TO CO-RELATE SERUM IRON AND SERUM FERRITIN IN RELATION TO CHOLELITHIASIS - A ONE YEAR CROSS SECTIONAL OBSERVATIONAL STUDY.** My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness

Name of the investigator:

Signature of the investigator:

ANNEXURE – II - PROFORMA

SERIAL NUMBER:

I. P. No

1. Name of the Patient:

2. Age:

3. Gender: 1. Male 2. Female

4: Occupation:

- Unemployed
- Unskilled
- Semi-skilled
- Skilled
- Professional

5.Education:

- Illiterate
- Primary (1^{st}-7^{th} std)
- High school (8^{th}-10^{th} std)
- Intermediate
- Degree and above

6. Socio-economic status:

- Low
- Middle
- High

Data collection instrument:

1. Duration of Pain –

2. Location of pain-

- Right lower quadrant
- Left lower quadrant
- Right upper quadrant
- Left upper quadrant

3. Mode of onset-

- Spontaneous
- Insidious

4. Associated symptoms-

- Fever
- Pain
- Vomiting
- Nausea

5. Medical history:

- Diabetes mellitus
- Hypertension
- Asthma
- CVD

Examination:

1

Height	Weight	Pallor

2.

Pulse rate	Blood pressure	Temperature	Respiratory Rate

3. Per abdomen examination

1) Point of tenderness-

2) Guarding

- YES
- NO

3) Rigidity

- YES
- NO

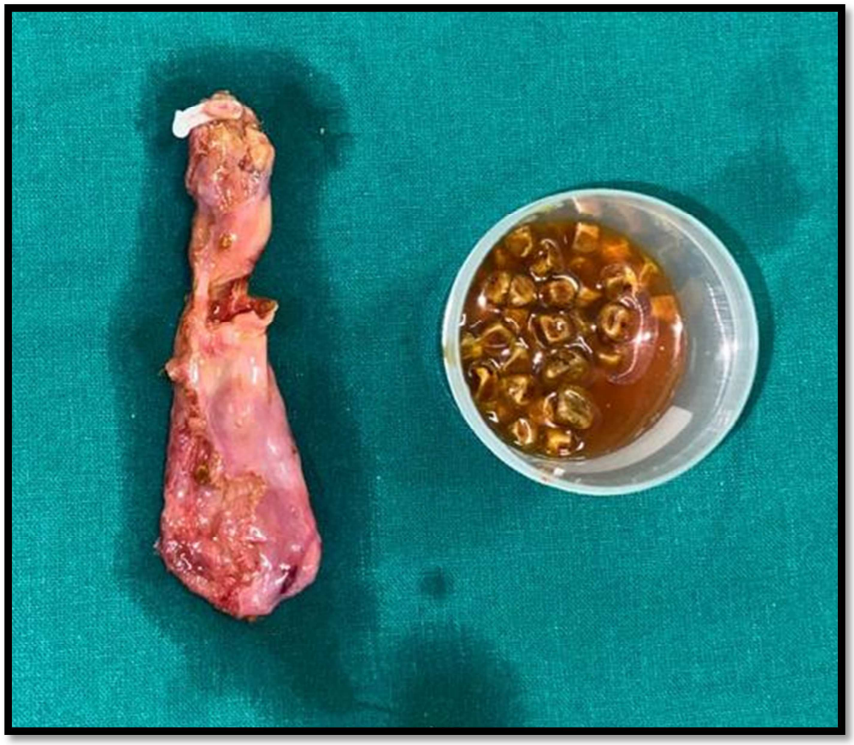
4) Bowel Sounds

- YES
- NO

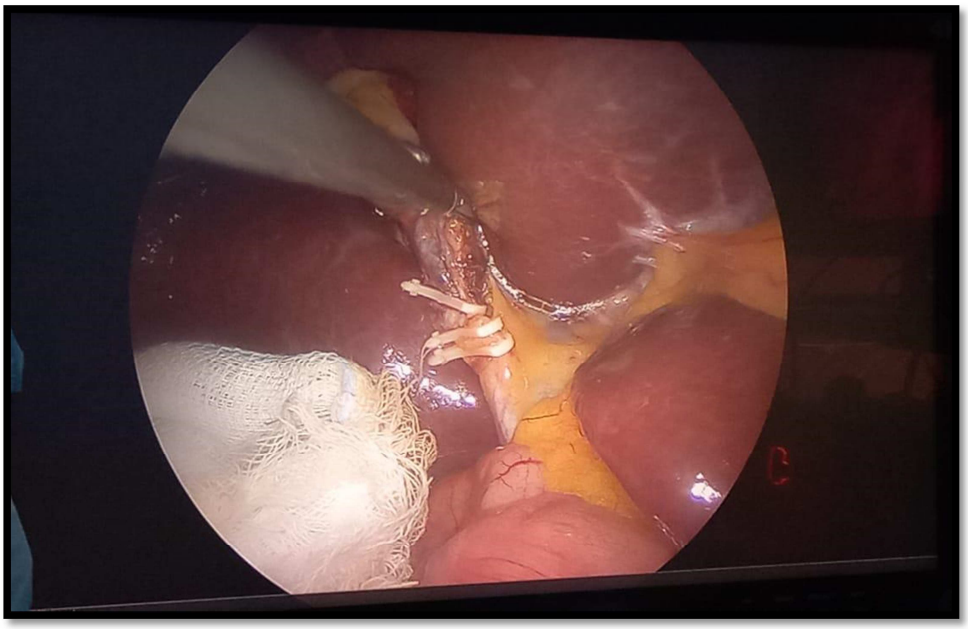
INVESTIGATIONS

Serum Iron	Serum ferritin	Haemoglobin

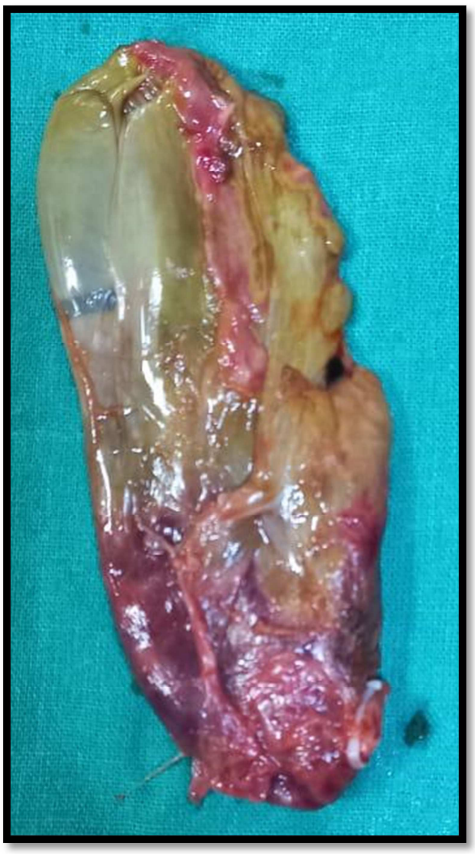
ANNEXURE – III - PHOTOGRAPHS



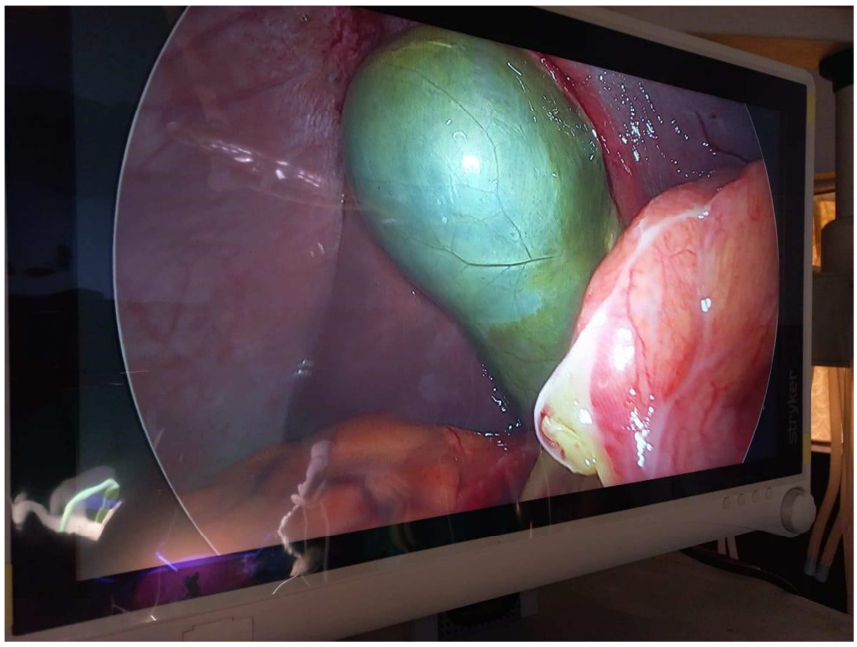
Photographs 1: Extracted Gallbladder with gallstones



Photographs 2: Intra-operative Clips applied to Cystic Duct



Photographs 3: Gangrenous gallbladder



Photographs 4: Intra-operative use of ICG dye for laparoscopic cholecystectomy

ANNEXURE – IV – MASTER CHART

Serial Number	IP Number	Age	Sex	Cholelithiasis	Serum Iron	Serum Ferritin	Haemoglobin	Anaemia
1	1131955	62	M	P	32	175.7	13.9	A
2	1133531	53	M	P	12	547.4	13.7	A
3	1149362	34	F	P	57	160.8	14.8	A
4	1152469	56	F	P	44	63.33	11.3	P
5	1151745	46	F	P	21	23.84	12.2	A
6	1154023	41	M	P	110	344.8	12	P
7	1154709	70	F	P	68	18.56	11.5	P
8	1156105	70	M	P	67	42.06	13.7	A
9	1154068	66	M	P	59	133.5	11.7	P
10	1156748	43	M	P	73	109.8	16.6	A
11	1166422	45	M	P	248	599.8	11	P
12	1176130	49	F	P	42	24.2	10.4	P
13	1174175	80	M	P	37	108.5	14	A
14	1184858	60	F	P	36	84.39	11.2	P
15	1184598	66	F	P	21	69.89	13.3	A
16	1184854	55	M	P	50	65.9	14.3	A
17	1187902	47	M	P	30	54.8	13	A
18	1188679	57	F	P	24	78.9	8.4	P
19	1189580	50	F	P	18	9.41	10.4	P
20	1190314	72	F	P	36	76	8.6	P
21	1191494	70	M	P	32	702.3	11.9	P
22	1192279	20	F	P	25	156	6.7	P
23	1157440	56	F	P	85	81.35	12.2	A
24	1156383	46	M	P	90	419	13.6	A
25	1156963	39	M	P	38	149.9	12	P
26	1156308	67	M	P	116	1556	10.1	P
27	1157634	36	F	P	33	4.66	10.7	P
28	1182982	30	F	P	56	98	12.8	A
29	1193646	57	M	P	43	66.9	14.7	A
30	1193503	26	F	P	19	37	10.3	A
31	1195025	64	F	P	31	73.4	11.9	P
32	1199332	73	F	P	28	170.6	10.6	P
33	1203873	60	M	P	38	126.8	13.5	A
34	1207010	40	F	P	30	96	11	P
35	1208665	41	F	P	13	45	9.6	P
36	1175505	46	M	P	29	469.1	10.9	P
37	10001227	54	F	P	14	134	9.6	P
38	10001437	65	F	P	15	663	10.6	P
39	10000461	54	M	P	21	2458	8.1	P

40	10000266	67	F	P	49	441	9.1	P
41	10016347	32	F	P	30	267	11.8	P
42	10010423	32	M	P	25	10	10	P
43	1207177	58	F	P	13	909	10.7	P
44	10002913	45	M	P	42	262	10.9	P
45	10002883	35	F	P	29	105	11.1	P
46	1209070	61	F	P	66	511	7.7	P
47	100002092	82	F	P	29	137	9.8	P
48	10005455	67	F	P	80	165	12.9	A
49	10006283	67	F	P	41	136	12	A
50	10007641	75	F	P	21	143	11.5	P
51	10009487	30	F	P	27	190	12.7	A
52	10009542	54	F	P	34	708	12.5	A
53	10010937	76	M	P	40	103	13.7	A
54	10009097	50	F	P	64	352	13.5	A
55	10009567	68	F	P	97	143.6	10	P
56	10012707	27	F	P	28	45	12.3	A
57	10024332	62	M	P	37	33.2	12.7	A
58	10028460	48	M	P	79	156	15.3	A
59	10029418	53	F	P	57	94.5	13.4	A
60	10029465	63	F	P	56	213	13.6	A
61	10041762	51	M	P	164	140	14.9	A
62	10040206	69	M	P	21	425	13.7	A
63	10044857	57	M	P	30	64	12.3	P
64	10045364	71	M	P	24	54	8	P
65	10046966	69	M	P	39	170	14.5	A
66	10047899	47	F	P	32	104	11.8	P
67	10048562	38	F	P	32	96	12.3	A
68	10049975	51	M	P	45	78.6	15.5	A
69	10050072	46	M	P	30	56	14.6	A
70	10047107	64	M	P	44	111	13.7	A
71	10049958	48	M	P	57	45	13.8	A
72	10050853	24	F	P	39	10.8	11.5	P
73	10051650	55	M	P	35	655	13.8	A
74	10053711	54	M	P	80	73.6	12.9	A
75	10054302	43	F	P	10	54.6	10.2	P
76	10053744	29	F	P	44	62.3	12	A
77	10055502	28	F	P	61	34.6	14.8	A
78	10055556	43	F	P	56	55.9	11.9	P
79	10054434	57	M	P	37	76.8	13.4	A
80	10055482	28	F	P	46	29.1	11.9	P
81	10056234	48	F	P	13	51.6	10	P
82	10057851	55	M	P	86	27	10.5	P
83	10056585	18	M	P	71	138	14.7	A
84	10056370	65	F	P	76	22.4	7.9	P
85	10060326	41	F	P	10	86	11.5	P